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SOME RELATIONS BETWEEN THE CONCENTRATION OF BLOOD CORPUSCLES IN VENOUS AND CAPILLARY BLOOD AND THE BLOOD PRESSURE OF DIABETIC PATIENTS*

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THE value of observation of the variations in the relative concentration of corpuscles in capillary and venous blood of diabetic subjects was suggested to us by the frequency of vascular disease and its sequelae in diabetics, and by the new point of view regarding the function of the capillaries brought out by Krogh[1] and his associates. Brown[2] has recently shown that in Raynaud's disease disturbed capillary tone, abnormal flow, or complete stasis may exist. The distribution of the blood corpuscles also has practical importance in relation to certain methods for determining blood volume, to capillary stasis such as occurs in traumatic shock, and possibly to the oxygen exchange in diabetes.

Methods.—Twenty-seven diabetic patients in the clinic of the New England Deaconess hospital on the service of Dr. E. P. Joslin including eleven men ranging in age from forty-six years to sixty-eight years and sixteen women ranging in age from twenty-eight years to seventy years, willingly served as subjects. In this group were two patients who had gangrene and suffered an amputation of the leg. Other patients were selected as having hypertension, hypotension, normal blood pressure, or acidosis. Counts of red and white corpuscles in venous blood and capillary blood of the same arm were made at 5:00 P.M. and at 7:30 A.M., just before the evening and morning meals. These hours were selected in order to eliminate, so far as possible, the effect of meals on the cell counts. The blood specimens were obtained at the same time or

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within a few minutes of each other. In withdrawing the venous blood, a tourniquet was applied only long enough to inject the needle into the vein; when the tourniquet was removed and the needle allowed to remain quiet within the vein for ten or fifteen seconds. Six c.c. of blood were withdrawn and a count was made from the last drop drawn. The blood was taken in clean, dry syringes and a red and white count made immediately. The capillary blood was drawn by pricking the tip of the finger with a three-sided needle without compressing the finger.

Hayem's solution was used for making the red count and a freshly prepared 1 per cent solution of acetic acid for the white count. All counts were made by us using the same pipettes and the same two counting chambers. The counting chambers were made by Bausch and Lomb with a Neubauer ruling. Pipettes and counting chambers were standardized by the Bureau of Standards and further checked by comparing counts from the same drop of blood. Samples of such check counts are given below.

	Pipette No. 2840		Pipette No. 3830	
(1)	157	153	154	155
	165	164	160	161
	159	144	156	154
	148	163	150	157
	Count 5,016,000 (R. R. W.)		Count 4,992,000 (H. F. R.)	
(2)	162	181	175	153
	160	164	173	165
	180	174	182	160
	177	162	157	186
	Count 5,440,000		Count 5,404,000	
(3)	164	167	158	152
	152	156	153	146
	153	150	154	164
	160	148	168	164
	Count 5,000,000		Count 5,036,000	

In taking the capillary blood, precautions were taken to have the tip of the finger dry. Because the pain and discomfort is less with venipuncture, it was customary to take the venous blood first. The time elapsing between venipuncture and the collection of the capillary blood was not more than three minutes, usually less.

Because of the possible influence of pain involved in drawing blood, blood pressure readings were taken before and after from the opposite arm. In some instances blood pressure readings were made on the arm from which blood counts were taken. Since the same technic was used in the morning and evening with special care to allow an interval between the taking of the blood pressure and the drawing of blood, these results are considered comparable with the others. We used a Baumanometer with a visible mercury column. The same instrument was used for all determinations. In taking the readings, the systolic pressure was that at which the sound of the pulse beat could first be heard as the pressure was released from the cuff. The diastolic pressure was taken at that point where the intensity of the sound first began to diminish and not at the point where it disappears. Three separate readings were taken in every instance and the lowest of the three taken as the proper reading.

TABLE I

RED AND WHITE CORPUSCLES IN CAPILLARY AND VENOUS BLOOD OF 23 DIABETICS

CASE NO.	SEX	AGE YEARS	DURATION OF DIABETES YEARS	EVENING						MORNING						ARTERIO-SCLEROSIS
				Blood Pressure Mm. Hg.		Venous		Capillary		Blood Pressure Mm. Hg.		Venous		Capillary		
						R. B. C.	W. B. C	R. B. C.	W. B. C.			R. B. C.	W. B. C.	R. B. C.	W. B. C.	
2946	M	46.2	1.8	87	58	3.7	6.7	5.0	6.8	77	53	3.8	5.5	4.3	4.0	0
1947	F	56.4	3.0	108	58	5.3	5.4	4.4	6.1	110	68	4.7	5.9	4.3	5.4	0
3032	F	49.6	6.1	118	60	4.9	8.4	4.5	9.9	112	60	5.2	7.5	4.9	8.1	0
3767	F	70.9	2.4	132	65	5.6	8.4	5.9	10.7	150	65	5.7		4.9	7.6	0
3689	M	47.6	1.8	100	63	4.3	5.2	4.3	5.8	103	67	4.4	3.4	4.4	3.4	0
3034	F	40.8	1.9	82	70	3.9	4.6	3.8	4.8	76	56	4.6	4.5	5.0	3.0	0
3195	M	68.7	12.9	130	70	4.7	7.5	4.8	8.2	126	71	3.4		5.3		++
2876	F	63.7	5.4	129	70	4.8	4.7	4.4	4.5	131	70	4.2	4.8	4.1	5.0	+
3210	F	64.3	0.6	170	75	5.2	10.1	4.0	5.8	165	70	4.8	10.5	4.8	12.8	+++
3194	F	50.3	1.0	114	74	4.5	5.4	5.6	5.8	108	80	4.8	4.2	4.8	5.7	0
3067	F	40.9	1.6	116	80	5.0	9.8	4.4	10.1	102	70	5.2	6.2	4.7	9.4	0
3666	F	28.8	0.6	120	78	5.6	7.2	4.6	10.3	110	68	3.5		5.4	7.0	0
3192	F	62.2	8.8	184	92	4.6	4.5	5.5	6.3	148	79	4.9	5.9	4.8	7.4	+
3193	F	55.0	8.0	152	81	5.3	4.9	4.7	6.0	121	73	5.2	5.6	5.2	5.2	+
2848	M	66.8	6.3	130	82	4.7	4.3	4.2	5.2	122	90	4.2	4.6	4.6	4.6	+
3898	M	67.0	0.3	173	81	5.3	7.5	6.2	8.9	130	70	5.6	8.0	5.4	6.4	+++
3728	F	39.6	1.2	153	95	5.3	7.3	5.9	6.4	148	91	5.3	5.7	6.0	5.4	0
3169	M	68.3	10.9	150	90	5.4	8.6	5.0	8.0	148	85	4.7	6.1	4.1	7.8	+
3944	F	57.8	2.1	213	92	6.0	9.3	5.9	9.8	194	90	6.4	8.2	5.1	8.7	+++
3728	F	39.6	1.2	113	92	5.5	7.1	5.3	6.0	107	74	5.3	5.8	5.7	4.8	0
2980	F	62.8	7.0	190	100	4.6	8.2	5.8	8.2	120	92	5.2	4.4	4.8	3.7	+++
2980	F	62.9	7.0	236	106	4.1	5.8	4.8	6.7	128	56	5.6	6.0	4.8	6.9	+++
2937	F	48.6	0.8	182	110	5.5	10.9	6.9	10.5	174	110	6.6	8.1	6.1	6.2	0
1730	M	54.4	6.8	160	120	6.0		7.0		130	90	5.1	5.6	6.0	3.9	++

NOTE: Red counts are expressed in millions and white counts in thousands.

DISCUSSION OF RESULTS

1. We would first direct attention to the fact that all counts were made from blood from the arm. Had capillary blood from a toe been used there is little doubt that greater variations might have been found. This would have involved the factor of posture to a much greater degree, and we decided against making this comparison. In Table I is given a list of the patients by case number showing age, duration of diabetes, their maximum blood pressure morning and evening, and the corpuscle counts. This series is arranged in the order of their diastolic pressures. There does not seem to be any obvious relationship between the duration of the diabetes and the diastolic pressure. The case with the shortest known duration had rather a high diastolic pressure. Similarly age did not seem to be a dominant feature in determining the diastolic pressure.

Arteriosclerosis was evident in the peripheral arteries of eleven subjects in varying degrees designated by +, ++, +++. Case No. 2980 showed advanced vascular change in the eyegrounds. Case No. 3210, at autopsy one year later, was found to have calcification in the walls of the coronaries, aorta, and big vessels.

Our first consideration is the change in cell counts between night and morning. In the interpretation of cell counts, it is of importance to know what variation may be expected in counts made at different periods during the day. In Table II are shown the average differences in counts made just before supper and just before breakfast.

TABLE II

AVERAGE DIFFERENCES IN CELL COUNTS WITHOUT REGARD TO SIGN BETWEEN NIGHT AND MORNING

RED CORPUSCLES		WHITE CORPUSCLES	
Venous	Capillary	Venous	Capillary
544,000	592,000	1,455	1,888

If the counts are averaged, the plus and minus differences balancing each other, such striking variations disappear. The average red count in venous blood for the entire group of patients in the evening was 5,001,500 and in the capillary blood 5,110,000. In the morning the average red count was 4,950,000 in venous and 4,974,000 in capillary blood. The white counts in the evening showed practically no variations, being 6,813 in the venous and 7,187 in the capillary blood. In the morning the white cells numbered 5,701 in the venous blood, and 6.097 in the capillary blood. The blood pressure on the other hand showed a considerable fall. The average systolic pressure in the evening for the entire group was 145 whereas in the morning it was 125. The diastolic pressure averaged 86 in the evening and 76 in the morning. Thus, with a considerable change in blood pressures, there was a very slight change in red cell counts but a definite decrease in the white count.

In Table III are shown the average variations in cell counts compared with the variation in blood pressure. It is seen that on the average the variations in blood pressures do not correlate with any characteristic changes

TABLE III

VARIATIONS IN NUMBER OF RED CORPUSCLES COMPARED WITH DIURNAL CHANGES IN BLOOD PRESSURE

BLOOD PRESSURE		NUMBER OF CASES	EVENING		MORNING	
Change	AVERAGE MM. HG.		Venous	Capillary	Venous	Capillary
Rise in systolic	7	3	4,889,000	4,863,000	4,743,000	4,477,000
Fall in systolic	24	19	4,987,000	5,153,000	4,985,000	5,128,000
Rise in diastolic	7	4	5,066,000	4,711,000	4,999,000	4,710,000
Fall in diastolic	14	14	4,899,000	5,144,000	4,810,000	5,146,000

Cases where no changes in blood pressure occurred are omitted.

in the red cell count. The variations in the counts between morning and evening are on the average not large enough to be significant. However, if the patients are classified according to maximal diastolic pressures, then a very different picture is presented. Patients whose maximal diastolic pressure was 90 mm. of mercury or more are eight in number. The average systolic pressure was 195 and diastolic 104. The average capillary count in the evening was 5,865,000 and the venous red cells numbered but 5,172,000. In the morning the relation was reversed, the venous count being 5,414,000 and the capillary 5,200,000. Similarly with the white cells the capillary count was higher than the venous in the evening but lower than the venous in the morning. This same relation appears in case No. 2946, whose arterial tension and also pulse pressure was very low. Patients whose maximal diastolic pressure was less than 90 mm. of mercury did not show any such striking variations in cell counts. If we classify patients according to their systolic pressure, we have those whose systolic pressure exceeded 150 mm. and those whose systolic pressure was below 150 mm. of mercury. This group includes all those patients whose diastolic pressure exceeded 90 with four other cases and shows a higher capillary than venous count in the evening with reversal of this in the morning.

The high systolic pressure does not correlate with any constant variation in corpuscle counts. If, now, we attempt to compare the fall in blood pressure with changes in corpuscle counts, again we find no correlation. It seems clear that in these cases the variation in arterial tension does not influence the relation between the red counts in capillary and in venous blood. In this group of patients two features seem to disturb the relation between the corpuscle counts in capillary and venous blood; namely, low arterial tension and high diastolic pressure.

Cannon[3] found a high degree of capillary stasis in men suffering from traumatic shock. The capillary red count in sixteen of our twenty-seven cases was over six million. In eleven cases with low arterial pressure (systolic 52-102) and shock, differences of from 400,000 to 2,000,000 cells between the venous and capillary counts were observed. Cohnstein and Zuntz[4] noted that when blood pressure fell, after cutting the spinal cord, capillary stagnation occurred and the number of red cells in the venous blood fell rapidly (drop of 1,000,000 corpuscles in ten minutes). In our cases, the fall in pressure was probably too gradual to result in such marked changes. Case No. 2946 with blood pressure in the "shock zone" exhibited a degree of capillary stasis comparable with the cases of shock described by Cannon.

Mall and Welch[5] noted that when the mesenteric artery was in part occluded, with a fall in blood pressure, red cells jammed the venous system, then the capillaries; and finally hemorrhage occurred. This plugging of capillaries as a result of a stasis might result in less return of blood to the heart, of lessened output, and a lowered blood pressure. Such a vicious circle which would interfere with nutrition seems a possible explanation of some of the factors in gangrene in diabetic patients with low blood pressures. This same patient (No. 2946), although he did not have gangrene, exhibited in high degree the sluggishness and a lack of vigor in healing when he came to the hospital with hot water bottle burns of the feet. Another factor which may be compared in diabetes and in shock is the lowered oxygen content of venous blood. Grant[6] showed that the oxygen unsaturation of the venous blood of diabetics is much higher than that of normals. According to Krogh the capillaries open in oxygen want. This gives a slower flow and greater absorption of oxygen by the tissues. With the lowered blood pressure and the inadequacy of the oxygen delivery, there is a decrease of the metabolism; Cannon records a reduction of one-third in severe shock. In a group of forty-three diabetics, without thyroid disease, five patients with systolic blood pressure above 150 mm. Hg. had an average basal metabolism 8 per cent above normal. Thirty-two with systolic blood pressure between 100 and 150 had an average basal metabolism exactly normal; there were six cases with systolic blood pressure below normal standard whose average basal metabolism was 10 per cent below normal.

TABLE IV

THE EFFECT OF THE APPLICATION OF A TOURNIQUET ON THE CONCENTRATION OF RED CORPUSCLES IN VENOUS AND CAPILLARY BLOOD*

CASE NO.	RED CORPUSCLES				BLOOD PRESSURE MM. Hg.
	BEFORE	AFTER			
		30 SECONDS	3 MINUTES	4 MINUTES	
	Millions	Millions	Millions	Millions	
Ba ------Cap.	5.6	5.6	4.8		120/80
Ven.	5.4		6.3		
McK -----Cap.	4.3	3.5	3.8		80/60
Ven.	4.3			5.3	
McK ---- Cap.	4.1	3.3		3.8	82/60
Ven.	3.6			4.3	
Lou ------Cap.	6.2	5.6	5.8		114/68
Ven.	6.2		7.0		
Ha ------Cap.	4.1	3.7		4.1	202/104
Ven.	3.6			4.3	

*The tourniquet was applied tightly enough to cause distention of the vein but not to shut off the arterial supply.

Patient No. 3034 does not show the same relationship in spite of a low arterial pressure. This may be explained in part by the fact that she had had acidosis and had been given large amounts of fluid. The group of cases, with high diastolic pressure and capillary stasis in the evening and a change in this relation in the morning, illustrate another mechanism producing capillary stasis. Gisell (quoted by Cannon) showed that after a reduction of blood volume but before the blood pressure fell, there might be a fall in the oxygen content due to constriction of the peripheral vessels. We may reason that in

these cases of high diastolic pressure there is an abnormal tension of the small arterioles with a compensatory dilatation of the capillaries under the stimulus of greater oxygen need during the day time. During the night, with a fall in pressure, this stasis is in part or wholly dissipated.

In contrast with the effects of change in arterial pressure Table IV shows the effects on the concentration of red corpuscles produced by variations in the venous pressure. It is seen that when a tourniquet is applied around the arm tightly enough to prevent the venous return, marked variations in the red counts occur. At the end of thirty seconds the capillary count falls, and at the end of three minutes it tends to rise a little but still to be much lower than the concentration in the venous blood. The obvious explanation seems to be that the blood plasma leaves the venous vessels, giving a relative concentration in the veins. The reduction in capillary count may be due to dilatation of the capillaries and dilution.

The passage of plasma into the tissue spaces would seem comparable to edema occurring in heart failure with venous engorgement. One might expect a concentration of red cells in the capillary blood equal to that in the venous, on the theory that the increased venous pressure would produce a flow of plasma from the capillaries into the tissues. However, these five cases show clearly opposite effects in venous and capillary blood. It suggests also that a relation may exist between disease of the finer vessels and effects produced by change in venous pressure. Thus, in the case of Mr. McK, who had marked edema with low blood pressure, the most marked reduction in capillary count was observed. This suggests that red cells may leave the capillaries in such cases, or else that certain of the capillaries under these conditions admit plasma but not corpuscles. In conditions of edema or of impending edema the capillaries or small veins may be peculiarly sensitive to changes in the venous pressure. At any rate, the subject merits further clinical study.

SUMMARY

1. The relative concentration of corpuscles in capillary and venous blood of twenty-seven diabetic patients in the morning and evening is compared.

2. Variations in arterial tension between these periods do not correlate constantly with any change in the corpuscle concentration.

3. In certain patients, with high diastolic blood pressure, the capillary count (red) was higher than the venous red count at night but lower in the morning. This reversed relation suggests capillary stasis at the end of the day and was more marked in patients with obvious vascular disease.

4. Increases in venous pressure produce the contrary effect; namely, high venous count and low capillary count.

5. It is suggested that further study is desirable. Comparisons of the relative concentration of corpuscles under varying conditions of arterial and venous pressure by means of the hematocrite should be made in cases of rheumatic heart disease, vascular hypertension and edema of cardiovascular origin.

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SEPARATION OF AN INTERNAL SECRETION OF THE PARATHYROID GLANDS*

By Louis Berman, M.D., New York City

IN MAY, 1924, I submitted the following statement to the Society for Experimental Medicine and Biology for its meeting in New Haven, Conn. This statement was published in the proceedings of the society for that month:

"Parathyroid glands from oxen were extracted with acidified alcohol. Lipins and proteins were removed from the extract. Crystalline material was obtained, by concentration, which increases definitely the calcium content of the blood when dissolved in Ringer's Solution and injected into the circulation. Its effect upon the calcium content of the blood has been compared with those of adrenalin, pituitrin and insulin. Whether the crystalline material is the active calcium mobilizing substance in the gland or merely a carrier of it in the preparation remains to be determined. Its separation is additional evidence in favor of the view that the parathyroid glands secrete a hormone that influences the calcium content of the blood."

Before this statement appeared in print, aqueous acidic solutions of this calcium mobilizing substance were obtained, and experiments were carried out to determine their effects upon parathyroidectomized dogs, cats, and white rats. The results obtained can be summarized as follows:

1. Sick parathyroidectomized animals presenting symptoms varying from malaise, anorexia, hyperpnea to tremors and tetany were relieved of their symptoms. Instead of lying about listlessly they walked about and responded to food and stimulation like normal animals. Animals were kept alive by successive daily injections. When the injections were stopped, the animals died.

2. All of the animals that presented symptoms showed a decrease in the blood calcium. After the injections and concomitantly with the improvement in their symptoms, the blood calcium increased. When the blood calcium decreased, there was a reappearance of the symptoms.

3. All of the animals which presented symptoms showed an increased electrical irritability of the nerves to galvanic stimulation. After the injections and concomitantly with the improvement in the symptoms and the increase in the blood calcium, there was a decrease in the electrical irritability.

*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.
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4. Injections made three hours after parathyroidectomy prevented the retention of the phosphate in the body which is registered by a marked decrease in the phosphate excreted in the urine. These results therefore show that protein-lipin-free solutions made by extraction of parathyroid glands with alcoholic solutions of acid (which were first used) or with aqueous solutions of acid (which were used immediately after the extractive efficacy of alcoholic solutions of acid had been established) will prevent or relieve the known cardinal effects following removal of the parathyroid gland, namely, the retention of phosphate, the decrease of blood calcium, the increase in electrical irritability of the nerves, the muscular hyperirritability and the various other symptoms. These results established the presence in the extracts of an internal secretion of the parathyroid glands since all the other criteria of substitution therapy are satisfied. It is therefore most gratifying to find that Collip (*Journal of Biological Chemistry,* April, 1925) has obtained, by the use of acid, extracts from the parathyroid glands which relieve the symptoms of parathyroidectomized animals as well as raise the level of the blood calcium in normal animals. His report, limited, it is true, to the effect upon the blood calcium and the symptoms, corroborates our findings.

We have been using the parathyroid extracts, found active in animals, in various clinical conditions associated with parathyroid insufficiency and a lowered blood calcium since May, 1924. The results of the observations will be reported in another publication.

THE ANTICOMPLEMENTARY REACTION OF BLOOD SERUM OR OF SPINAL FLUID*

By Arthur H. Sanford, M.D., Rochester, Minnesota

IN the discussion of Kolmer's paper presented before this Society in 1924 the subject of the anticomplementary reaction was introduced. It was pointed out that rarely fresh serum, uncontaminated by bacteria and with no change due to age, would fix complement without antigen so that there would be no hemolysis in the control tube, and a definite report could not be made. Though indeed rare, the phenomenon is, I believe, of enough importance to be considered further, with special reference to the Kolmer technic.

An analysis of the 46,549 tests, made in the Mayo Clinic in 1924, shows that either serum or spinal fluid was anticomplementary in ninety-six instances, that is, in slightly more than two in each thousand tests. The tests were all set up the same day that the blood or spinal fluid was drawn. Very little has been written in recent years about the fixation of complement without antigen. Soon after the reaction of Wassermann, Neisser, and Bruck had attracted general attention, a few authors reported their experiences in this respect. Ballner and von Decastello made a comparative study of the Wasser-

*From the Section on Clinical Pathology, Mayo Clinic.
Read before the American Society of Clinical Pathologists, Philadelphia, May, 1925.

mann reaction by the cattle blood hemolytic system and the sheep cell system. Their paper contains little of interest today, except their statement that between the positive reaction in syphilitic and that in probable nonsyphiltiic blood, there seemed to be a consistent difference in using the cattle blood system. In syphilis complement binding occurred only after the addition of organ extract, at least it was definitely strengthened by it, while in nonsyphilitic blood the lack of or addition of extract made no difference in the intensity of the fixation. They called an anticomplementary serum "autotrope serum." When sheep's blood amboceptor was used the autotrope serums reacted as syphilitic blood. When extract was added hemolysis was inhibited, and without extract more or less definite hemolysis occurred. The cattle blood system seemed to make it possible to distinguish a specific from a nonspecific reaction. The authors mention tuberculosis of the lungs and kidneys, pneumonia, carcinoma, myeloid leucemia and cardiac disease with compensation as nonsyphilitic diseases exhibiting this phenomenon of false fixation by so-called autotrope serum.

Hecht next discussed this subject of anticomplementary serum (eigenhemmendes Serum). He maintained that practically all blood has natural sheep amboceptor, that practically all blood has complement fixing substances, and that nearly all have complement. He believes that lack of balance of these substances causes fixation without antigen.

Ehrmann and Stern the next year quoted Ballner and von Decastello's work, but believed that the anticomplementary reaction might be characteristic of syphilis, as they stated that syphilitic serum in large quantities would fix complement without antigen. They stated that syphilitic serums reacting positively in quantities of 1 c.c. would frequently fix complement without organ extract, while nonsyphilitic serums did not show this phenomenon. Hecht controverted this statement by Ehrmann and Stern by asserting that he did not consider this reaction positive for syphilis. He showed that, in one case of lupus verrucosus, the anticomplementary reaction of the serum was stronger than in two cases of syphilis with anticomplementary serum, and that normal serum mixed with sheep's blood fixed complement in quantities of 1 c.c.

Only one other paper of these early writers need be mentioned, that of Trinchese, who reported a series of 7,000 Wassermann reactions and observed autoinhibition once in 500 serums, or in practically the same ratio as was observed in the Mayo Clinic in 1924. He stated that clinical experience has shown that autoinhibition in the original Wassermann reaction occurs only in syphilitic serums or in serums suspected of being syphilitic, and that it depends on the presence of antigen in the syphilitic serum.

It is seen by these references to the literature that the anticomplementary reaction of serum was early recognized and that there was from the beginning much difference of opinion as to what it meant. Differences in technic might account partly for the varied results. There are no reports in the literature regarding anticomplementary reactions with the Kolmer technic, other than that published in the discussion of the paper referred to.

Analysis of the ninety-six reactions in our series has been made, and I regret to say that our conclusions cannot at this time throw much light on the

subject. While the number of reactions was ninety-six, the total number of patients was but fifty-eight, as there were many repeated tests. The serums of thirty-seven patients were anticomplementary one or more times. Not all of these patients in this group had spinal fluid examinations, but such spinal fluids as were examined were not anticomplementary. On the other hand, of the twenty-one patients whose spinal fluids showed one or more anticomplementary reactions, in no case did the serum give such a reaction, although several examinations of the blood of each patient were made. In other words, there was not a single instance of anticomplementary reaction of both blood and spinal fluid in the same patient. Whether this is of any significance cannot be determined in a small series. However, the anticomplementary reaction of spinal fluid has apparently attracted but little attention, according to other writers, although this condition must be met with by all serologists.

ANTICOMPLEMENTARY BLOOD SERUM AND SPINAL FLUID

There were thirty anticomplementary reactions in twenty-two cases diagnosed as nonsyphilitic, although it must be said that in some of these cases the likelihood of syphilis was not thoroughly investigated by the clinician, because there were no outward signs or history of the disease. The diagnoses of the noninfectious diseases in these twenty-two cases were: neurasthenia, two; menopause neurosis, one; psychoneurosis, one; general arteriosclerosis, one; achlorhydria, one; sacroiliac strain, one, and colloid goiter, one. The findings in these cases are apparently purely incidental and might possibly be attributed to some error in technic. There was also a group of cases of infection: conjunctivitis, one; retinitis proliferans, one; infectious hepatitis, one; chronic cholecystitis, one; arthritis, one; myocarditis, two; endocarditis, one; streptococcemia, one; and tubercular peritonitis, one. There were also three cases of malignant disease: myeloma, one; metastatic carcinoma, one; and lymphosarcoma, one. There was one case of splenic anemia.

From the foregoing list of diseases it may be definitely concluded that the reaction may occur rarely in patients in whom there is no history of syphilis and who are suffering from some other disease. The reaction occurred sixty-three times in thirty-three patients with syphilis. There were also three doubtful cases of anticomplementary reactions with a probable diagnosis of syphilis without syphilitic history. The reaction may then be considered suggestive of syphilis and I believe that a report of anticomplementary reaction should be considered by the clinician as of enough significance always to investigate thoroughly the possibility of syphilis. While many patients with syphilis in this series have had treatment of various sorts, there were others who most noticeably had not had treatment. The effect of treatment is not evident. The occurrence of the anticomplementary reaction can be illustrated in a report of two small groups of selected cases.

SELECTED CASES WITH ANTICOMPLEMENTARY BLOOD SERUM

CASE 1.—A man, aged forty-three, complained chiefly of painful joints for fifteen years. He had been married eighteen years, without children. His wife had syphilis. There was no definite history of syphilis, but he had had gonorrhea fifteen years before. The Kolmer test, March 15, 20 (two different samples of blood), 21, 22, and 24 (two

different samples of blood), 1924, was anticomplementary. The serum was sent to Kolmer, who reported it anticomplementary. The diagnosis was indeterminate but probably syphilis.

CASE 2.—A boy, aged twelve, complained chiefly of physical and mental weakness all his life. The father had had chancre on the lip four months before the birth of the patient, and the mother had had chancre on the genitalia two months before his birth. The Kolmer test on the serum had been 44 November 21, 1923, and was again 44 January 25, and May 2, 1924. The serum was anticomplementary April 2, and May 27, 1924. The spinal fluid had been 44444 November 26, 1923, and was 4444– January 28; 4442– April 3; 444– – April 30; 444– – May 14; and 444– – May 27, 1924. The diagnosis was cerebrospinal syphilis, juvenile taboparesis.

CASE 3.—A man, aged sixty, complained of having had a lump above the eye for one year. He had had syphilis twenty years before. With the Kolmer test the blood serum had been anticomplementary on four different occasions in 1923, and July 7, 1924. The diagnosis was tertiary syphilis.

CASE 4.—A woman, aged thirty-three, complained chiefly of having felt generally run down for several years. There was no definite history of syphilis. With the Kolmer test the blood serum was anticomplementary September 16, 18, December 22, and 23, 1924. The Kolmer test was 44 September 23, 24, and 25. The spinal fluid gave a negative reaction. The diagnosis was latent syphilis.

From the foregoing selected cases I have tried to show that in some instances it seems impossible to get anything but an anticomplementary reaction in repeating the Kolmer test with fresh serum inactivated the ordinary length of time. In other instances the anticomplementary reaction may be present one day and a few days later with a new titration of different reagents the reaction does not persist. However, the clearing of the control tube then makes the reaction very strongly positive.

SELECTED CASES WITH ANTICOMPLEMENTARY SPINAL FLUID

CASE 5.—A man, aged sixty, complained of confusion of ideas and forgetfulness, for an indefinite period. There was no history of syphilis although his wife had syphilis. The Kolmer test on the blood had been 44 July 11, August 10, 1923, and July 1, 1924. The Kolmer test of the spinal fluid was anticomplementary twice in 1923 and again July 2, 1924. The diagnosis was late syphilis of the central nervous system.

CASE 6.—A man, aged thirty-nine, complained of stomach trouble and gave a questionable history of syphilis. The Kolmer test of the blood was 44 July 28, and 31, 1924, but negative November 11. With the Kolmer test, the spinal fluid was anticomplementary July 31, September 25, October 9, and 23, 1924. (The spinal fluid has been anticomplementary once in March, 1925.) The diagnosis was late syphilis of the central nervous system.

CASE 7.—A man, aged forty-three, had complained of extreme nervousness for nine months. He had had a lesion on the penis fourteen years before, with no treatment. The Kolmer test of the blood was 44 January 2, 5, and May 22, 1924. The spinal fluid was anticomplementary January 5, and 4444– May 22, 1924. The diagnosis was syphilis of the central nervous system, paresis sine paresi.

CASE 8.—A man, aged thirty-four, had had syphilis twelve years before with much treatment. The Kolmer test of the blood was 44 January 4, 8, 9, February 7, June 17, and July 2, 1924. The Kolmer test showed that the spinal fluid was anticomplementary January 9, 23, February 6, 20, June 18, July 18, 1924. The diagnosis was syphilis of the central nervous system.

The Nonne-Appelt test on all of these spinal fluids showed an increase in globulin, and the lymphocyte counts were all above normal.

It would seem, then, that an anticomplementary reaction with spinal fluid is a more likely indication of syphilis than when it occurs with serum. This may be more apparent than real, inasmuch as there are fewer negative controls since spinal punctures are not made as often as venipunctures in non-syphilitic cases. However, there is always a large proportion of spinal fluids in every day's run of Wassermann tests that give negative results. Paresis and taboparesis, has been the diagnosis in so many of these cases in which the spinal fluid has shown anticomplementary reaction, that the syphilologists in the Mayo Clinic are beginning to feel that the anticomplementary reaction in the spinal fluid may be an indication of this disease. In any event the number of cases observed is undoubtedly too small to make this statement of any positive value.

Several attempts were made to learn something of the nature of the anticomplementary substance. While none of these has proved of much worth, I shall present them briefly in order to indicate the difficulties encountered in making such a study. It is, after all, part of the whole story of what constitutes the basis for any reaction in immunity.

In Table I is reported a series of titrations to determine the amount of anticomplementary substance in various serums with which there was no hemolysis in the control tube in the ordinary quantity used, namely 0.1 c.c. It will

TABLE I

TITER OF ANTICOMPLEMENTARY SUBSTANCE IN BLOOD SERUM

Complement in all tubes in double unit quantities

NUMBER	SERUM WITH ANTIGEN					SERUM WITHOUT ANTIGEN				
	0.1 c.c.	0.05 c.c.	0.025 c.c.	0.005 c.c.	0.0025 c.c.	0.1 c.c.	0.05 c.c.	0.025 c.c.	0.005 c.c.	0.0025 c.c.
1	++++	++++	++++	−	−	+++	−	−	−	−
2	++++	++++	+++	−	−	+++	+	−	−	−
3	++++	++++	++++	++++	++++	++++	++++	++++	−	−
4	++++	+++	−	−	−	++++	++	−	−	−
5	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
6	++	+	−	−	−	+	−	−	−	−
7	++++	++++	+	−	−	++++	+	−	−	−
8	++++	++++	++++	−	−	+++	++	−	−	−
9*	++++	++++	+++	−	−	+++	++	−	−	−
10	++++	++++	++++	++	+	++	+	−	−	−

*Same as No. 8, two days later.

TABLE II

TITER OF ANTICOMPLEMENTARY SUBSTANCE IN BLOOD SERUM

Fixing power in higher concentration of complement

NUMBER	SERUM WITH ANTIGEN*					SERUM WITHOUT ANTIGEN*				
	CONCENTRATION OF COMPLEMENT					CONCENTRATION OF COMPLEMENT				
	5X	4X	3X	2X	1X	5X	4X	3X	2X	1X
1	−	++	+++	++++	++++	−	−	−	−	++
2	−	−	++	++++	++++	−	−	−	++++	++++

*Quantity of serum in each tube, 0.1 c.c.

be seen that in only one instance did the titration of the anticomplementary substance exhibit the same degree of fixation as where the serum was used with antigen. Ruediger, in the discussion of Kolmer's paper, said that he was able to get rid of the anticomplementary substances by reheating at intervals of two or three days, or by running a control tube for every antigen tube, using the human serum in decreasing quantities, because in such cases the specific fixation will always occur in dilutions in which the anticomplementary property cannot be detected. This is evidently true in the small series that I was able to run. I found also that blood saved in the ice box for three days was no longer anticomplementary. Fresh serums may account, to some extent, for the anticomplementary reactions in this series.

In Table II, I have shown the results with two other serums that were titrated in another manner, namely, by determining the fixing power in higher concentrations of complement. I have done this several times when I have been interested in the amount of syphilitic antibody present in the serum, determining the quantity of complement that could be fixed with a constant quantity of patient's serum, rather than determining how little of the patient's serum would fix a constant quantity of complement. It is shown that the anticomplementary substance without antigen will not fix as high a concentration of complement as will the same serum when used with antigen.

TABLE III

TITER OF ANTICOMPLEMENTARY SUBSTANCE IN SPINAL FLUID

Complement in all tubes in Kolmer double unit quantities

NUMBER	SPINAL FLUID WITH ANTIGEN					SPINAL FLUID WITHOUT ANTIGEN				
	0.5 c.c.	0.25 c.c.	0.125 c.c.	0.0625 c.c.	0.03125 c.c.	0.5 c.c.	0.25 c.c.	0.125 c.c.	0.0625 c.c.	0.03125 c.c.
1	++++	++++	++++	++++	+	++++	+++	–	–	–
2	++++	++++	++++	+++	–	+	+	–	–	–
3	++++	++++	++++	–	–	++++	+	–	–	–

TABLE IV

TITER OF ANTICOMPLEMENTARY SUBSTANCE IN SPINAL FLUID

Higher concentration of complement

NUMBER	SPINAL FLUID WITH ANTIGEN* CONCENTRATION OF COMPLEMENT					SPINAL FLUID WITHOUT ANTIGEN* CONCENTRATION OF COMPLEMENT				
	5X	4X	3X	2X	1X	5X	4X	3X	2X	1X
1	+	++++	++++	++++	++++	0	++	+++	++++	++++
2	Not enough fluid					–	++	+++	++++	++++
3	+	++++	++++	++++	++++	–	–	–	–	+++

*Quantity of spinal fluid in each tube, 0.5 c.c.

Tables III and IV show in like manner the titer in anticomplementary substance as found in spinal fluid. In Table III the titration of spinal fluid is against a constant quantity of complement both with and without antigen; in Table IV a constant quantity of spinal fluid, namely, 0.5 c.c., was used, and the degree of fixation with higher concentrations of complement up to five times the normal amount, both with and without antigen is recorded.

Hecht's suggestion regarding the cause of "autoinhibition" prompted an attempt to titrate the amount of complement in anticomplementary serum. Table V shows the result of one of these experiments. A criticism of the result is apparent. While there was no hemolysis with any quantity of anticomplementary serum, the conclusion cannot be drawn that there is no complement present in such serum, inasmuch as there would be fixation of complement by the anticomplementary substance; no satisfactory experiment could be devised for demonstrating whether or not an anticomplementary serum really contains complement.

It was suggested by Trinchese that the property is due to the presence of antigen in the serum. Table VI shows a small series in which the anticomplementary serum was titrated for antigenic properties. The control tubes with-

TABLE V

COMPLEMENT TITRATION OF HUMAN SERUM

SERUM	SALT SOLUTION 0.0 c.c. SERUM (1/10) 1.0 c.c.	SALT SOLUTION 0.2 c.c. SERUM (1/10) 0.8 c.c.	SALT SOLUTION 0.4 c.c. SERUM (1/10) 0.6 c.c.	SALT SOLUTION 0.6 c.c. SERUM (1/10) 0.4 c.c.	SALT SOLUTION 0.8 c.c. SERUM (1/10) 0.2 c.c.	REMARKS
Anticomplementary	O	O	O	O	O	Amboceptor 1/2000
Syphilitic	H	H	H	±	O	0.5 c.c. each tube
Negative	H	H	H	H	O	0.5 per cent sheep cells
Negative	H	H	H	H	O	0.5 c.c. each tube
Negative	H	H	H	H	O	Incubation one hour
Negative	H	H	H	H	O	

H = hemolysis; ± = incomplete hemolysis; O = no hemolysis.

TABLE VI

TITRATION FOR ANTIGENIC PROPERTY OF ANTICOMPLEMENTARY BLOOD SERUM

SERUM*	ANTICOMPLEMENTARY SERUM				
	0.1 c.c.	0.05 c.c.	0.025 c.c.	0.005 c.c.	0.0025 c.c.
Control (no serum added)	++++	++++	–	–	–
1. Syphilitic	–	–	–	–	–
2. Syphilitic	–	–	–	–	–
3. Syphilitic	–	–	–	–	–
4. Syphilitic	–	–	–	–	–

*0.1 c.c. in each tube.

TABLE VII

TITRATION FOR ANTIGENIC PROPERTY OF ANTICOMPLEMENTARY SPINAL FLUID

	ANTICOMPLEMENTARY SPINAL FLUID				
	0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.
Control (no serum added)	++++	++++	++++	+++	–
Syphilitic spinal fluid, 0.5 c.c.	++++	++++	++++	+++	–
1. Syphilitic serum, 0.1 c.c.	–	–	–	–	–
2. Syphilitic serum, 0.1 c.c.	–	–	–	–	–
3. Syphilitic serum, 0.1 c.c.	–	–	–	–	–
4. Syphilitic serum, 0.1 c.c.	–	–	–	–	–
5. Syphilitic serum, 0.1 c.c.	–	–	–	–	–
Pooled syphilitic serum, 0.1 c.c.	–	–	–	–	–

out syphilitic serum showed that this particular serum was anticomplementary in quantities of 0.1 c.c. and 0.05 c.c., but when syphilitic serum was added there was no fixation of complement.

In Table VII the results of titration of anticomplementary spinal fluid for antigenic property are recorded. This spinal fluid was anticomplementary in quantities down to 0.2 c.c. When syphilitic serums were added there was no fixation of complement. However, when a syphilitic spinal fluid was added to each tube in 0.5 c.c. quantities, there was the same degree of fixation that had been manifested by the anticomplementary spinal fluid when used alone. It is doubtful whether this is an evidence of antigenic action. Fixation is probably due to the anticomplementary substance in the spinal fluid, uninfluenced by the addition of syphilitic spinal fluid. It is a little difficult to conjecture, however, what has happened to the anticomplementary fluid when syphilitic serum is added to the various tubes and no fixation of complement occurs. Further study must be made of this type of reaction to really make clear the significance of this phenomenon.

At present a study is being carried on in our laboratory comparing precipitation tests on serums that have been shown by the Kolmer test to be anticomplementary. As complement plays no part in this test it might be argued that an anticomplementary reaction could not occur, although it must be admitted that the same substance that is fixing complement, whether with or without antigen, may also be responsible for the flocculation phenomenon described by Kahn, Vernes, Meinecke, Sachs-Georgi, and other advocates of precipitation tests for syphilis.

SUMMARY

An anticomplementary reaction of fresh serum, or spinal fluid, occurs with the Kolmer modification about twice in each thousand tests. This reaction is not specific for syphilis, although it occurs more often in syphilitic than in nonsyphilitic cases. In fact, its occurrence with spinal fluids may be indicative of severe syphilitic involvement of the central nervous system. The nature of the anticomplementary substance has not been fully determined. It does not seem to be due to natural antigen in the serum, or spinal fluid itself. It is transient in character in some cases, and in others is found constantly. Quantitatively it is not as active as is the complement-fixing property of syphilitic serum when antigen is added. Flocculation tests on anticomplementary serums may throw some further light on this interesting subject.

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A STANDARDIZED WASSERMANN REPORT*

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THE purpose of this paper is to try to induce laboratory workers to state the results of their Wassermann tests in simpler language which will be better appreciated by clinicians.

The Wassermann test is made differently in almost every laboratory. Different degrees of accuracy and sensitivity result, each worker having his own idea as to the desirable degree of sensitivity for his results to be diagnostic of syphilis. The same worker may argue against the reporting of complete fixation as positive on the grounds that in stating that the test is positive he would be making an improper interpretation for the clinicians.

It is practically impossible to cause the majority of workers to adopt any definite technic because of differences of opinion and technical facilities. Near one extreme we have those diminishing few who, apparently from choice, attempt to fix complement by plain syphilitic liver antigens approximately in one hour at 37° C., and near the other extreme we have a large group of those with good technical facilities who do their fixation with purified lipoids for approximately twenty hours at temperatures lower than 8° C.

The reports to the clinician are given in various terms which suggest accuracy, probably the commonest terminology is plus-four as indicating complete fixation, plus-three as about three-fourths fixation, plus-two as one-half fixation, and plus-one as one-fourth fixation. The clinician is led to believe by such a report that a plus-two serum is necessarily just half as strong in Wassermann bodies as a plus-four serum which apparently contains the maximum amount of Wassermann bodies. The clinician is told of the relatively unimportant distinctions between a serum which is plus-three and one which is just plus-four, but he is deceived by being given the same report of plus-four on two serums, one of which may be sixteen or more times as strong as the other. If the clinician is told merely that both of these plus-four serums are positive he does not receive any suggestion that they are of exactly the same strength.

In the past, when the weak antigens produced less than complete inhibition in secondary syphilis, there was a greater difference than now between a plus-two and a plus-three serum and it may have seemed of some value to report such differences. No difference would be noted with such serums tested today by the more sensitive methods (except by quantitative tests) as they both would show complete inhibition. There is also less difference today between plus-one and plus-two serums. This difference continues to grow less with each increase in sensitivity.

*Read before the Fourth Annual Meeting of the American Society of Clinical Pathologists, Philadelphia, Pa., May 20-23, 1925.

This system of recording complete fixation in a single tube by four-plus is a common method in this country in private laboratories but, in the navy, a system is used in which two-plus signifies a complete reaction. Then there are other methods particularly in state laboratories where the results in two dilutions of serum are added together; plus-two, or complete fixation, in two tubes is recorded as plus-four, and a plus-two might mean plus-one in each of two tubes, or complete fixation in one tube with no fixation in the other.

These various hieroglyphics are understood by the individual serologist; they are useful in recording results according to his own private formula, but they should not be allowed to mislead the clinician.

The ordinary qualitative report is of sufficient assistance to the clinician in discovering the presence of syphilis. A report of a negative, positive, or doubtful result is all that is necessary for that purpose. Partial inhibition of hemolysis should be reported as doubtful; probably only complete fixation should be reported as positive. The clinician should not be given any spuriously quantitative reports. The only quantitative statement which should have any place on a qualitative report should be one giving a coefficient of sensitivity. It might be stated on the report that the method used is positive to a certain number of "A.S.C.P." or perhaps "Wassermann" units. Then the clinician will be better able to evaluate the evidence of a negative Wassermann report.

A quantitative test should be available to those who are giving treatment. An arbitrary numerical standard should be adopted by the American Society of Clinical Pathologists to be called the "A.S.C.P." unit which should correspond approximately in different laboratories.

Without going into more detail than is necessary to show the feasibility of this plan, I shall outline a simple procedure by which it is possible to standardize the results of the test. The details should be determined by a committee of this society.

Strongly positive serum is dried by spontaneous evaporation at room temperature in watch crystals. One-half a milliliter of serum is placed in each of six or more watch crystals. After the serum has dried, the crystals are stored in a desiccator for two weeks or more. The serum should be of such strength that it will give a positive reaction in a dilution of one in four or more with a certain definite standard method. One watch crystal of this dried serum is redissolved in the calculated amount of distilled water and salt solution to make a one in four dilution and used as though it were pure serum. Then this diluted serum is tested quantitatively in the same and greater dilutions to determine which dilution gives complete fixation according to a definite standard technic. The coefficient of sensitivity of this standard method naturally would be considered as 1.

The dilution which gives just complete fixation shall be considered arbitrarily as being an undiluted standardized four unit serum. In other words, the standard method gives complete fixation with a serum containing just four Wassermann units. A more sensitive method might give complete fixation with a further dilution of the four unit serum to one in four. Then

the more sensitive method would have a coefficient of sensitivity of 4 as it would be four times as sensitive as the standard method with a coefficient of 1.

If standardized four unit serum is used, the simple rule for obtaining this coefficient is to divide the coefficient of the standard method (1) by the further dilution of the serum necessary to obtain just complete fixation with the new method (or by the proportionate reduction or increase in dosage from the usual in the new method).

Example 1: A new method requires further dilution of standard serum to 1 in 4. $1 \div \frac{1}{4} = 4$. Therefore the coefficient of sensitivity is 4. This coefficient shows the new method to be four times as sensitive as the standard method.

Example 2: The usual dose of serum in a new method is 0.5 c.c. of serum, but instead of further dilution of the standard serum being used a dose of 0.125 c.c. is found to give just complete fixation.

$$1 \div \frac{0.125}{0.5} = 4.$$

Example 3: A new method does not give complete fixation with 0.5 c.c. the usual dose used, but requires double that dose or 1 c.c.

$1 \div \frac{1}{0.5} = 0.5$ Therefore the coefficient of sensitivity of the new method is 0.5. The new method is half (0.5) as sensitive as the standard method.

If serum of known strength other than four unit strength is used, the same rule will apply, but the result must be multiplied by the proportion existing between the strength of the standard serum and of the serum to be used.

Example: Strength of serum used 32. Usual dose of serum in new method 0.5 c.c., required dose 0.125 c.c.

$$1 \div \frac{0.125}{0.5} = 4.$$

$4 \times \frac{4}{32} = 0.5$, the coefficient of sensitivity.

Any method giving constant results, when its coefficient of sensitivity has been determined can be used for standardizing serum for standardizing other methods. To find the strength of a serum in units, the coefficient of sensitivity is divided by the dilution necessary (or by the usual dose into the dose necessary) to just give complete fixation, and the product is multiplied by 4. The product is multiplied by four, because a serum is considered to be of 1 unit strength when it contains one-fourth the amount of Wassermann substance necessary to give complete fixation with a method with a coefficient of 1. The serum is then diluted as indicated to make it a standard four unit serum.

Example 1: Coefficient of method is 2. Necessary dilution of serum is 1 in 4. $(2 \div \frac{1}{4}) \times 4 = 32$. Therefore the unknown is a 32 unit serum. This serum if diluted to 1 in 8 would be a standard four unit serum.

Example 2: Coefficient is 2. Usual dose of serum 0.5, necessary dose 0.125.

$$(2 \div \frac{0.125}{0.5}) \times 4 = 32.$$

Where there is any local difficulty different laboratories can easily check results by exchanging standardized dried serum.

The method used for standardizing should be one which is quite simple, available to workers with limited facilities, and one which eliminates as far as possible any personal equation. A serum is diluted at least one in four and greater dilutions are made until some dilution gives just complete fixation with the following technic. That liquid is considered as containing four units, is called the standard four unit serum, and is used for standardizing any other method.

In the standardizing method the serum is inactivated for one-half hour at 56° C. The dosage of the standard four unit serum is 0.1 c.c.

The complement is used on the day the test is made but aged by being placed in the 37° C. incubator for one hour before separating the serum. After separating the serum it is diluted one in twenty, and 0.5 c.c. is used as the dose. A definite dose of complement is used unless the complement is found to be quite inactive when it is discarded. The definite dose is used instead of varying it by means of the hemolytic activity. In my opinion attempting to estimate the fixability of complement by the hemolytic activity will give more variability than the use of a fixed dose of guinea pig serum. This is one of the details which should be determined by a committee and I am suggesting this method in some detail merely as a framework and not to provoke controversy.

The antigen is next added in a dose of 0.2 c.c. of a one in ten dilution. The antigen should be prepared after the method of Neymann and Gager.[1]

Pulverized heart muscle (Difco brand is suitable) is completely extracted with ether to remove cholesterol and certain substances of variable composition. The heart, free from ether, is added to absolute alcohol in the proportion of 20 grams of heart to 100 c.c. of alcohol. This mixture is placed in the incubator at 37° C. for one week. The alcohol is poured off or filtered off and cholesterol is added to make a 0.2 per cent solution. This antigen, because of the low cholesterol content, is diluted slowly.

Fixation is accomplished by leaving the tubes in a water-bath at 37° C. for one hour.

Sheeps corpuscles, not over twelve hours' old, are next added in a dosage of 0.2 c.c. of a 6.25 per cent suspension.

Two units of amboceptor contained in 0.2 c.c. of solution are next added and the tubes shaken and returned to the water-bath. The readings are made as soon as the cells have settled sufficiently, for the total absence of hemolysis in some tubes to be clearly visible.

Some other Wassermann technic may be found more constant in its results or otherwise more desirable, but the method suggested will serve the purpose until a better one can be determined upon. Slight variations in numer-

[1]Neymann and Gager: Jour. Immunol., 1917, ii, 573.

ical results can be eliminated if necessary by repeating the test a number of times on the serum to be standardized and averaging the results.

The routine test for diagnosis should be the qualitative test, the result of which should be given as negative, doubtful, or positive and accompanied by the coefficient of sensitivity for the method used.

The routine test for observing the effects of treatment should be the quantitative test, the result of which should be expressed in "A.S.C.P." units.

THE PRESENT STATUS OF KOLMER'S COMPLEMENT-FIXATION TEST FOR SYPHILIS AS ESTABLISHED BY A CRITICAL COMPARISON WITH NUMEROUS OTHER METHODS*

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THE value of any new laboratory procedure or any modification of a method already in use can only be ascertained by comparative trial, and the more critical the comparison and the more extensive the trial the more significant are the ultimate conclusions.

Of all the methods which have been proposed for the adaptation of the complement-fixation test to the serologic diagnosis of syphilis there are none which have been subjected to comparative, critical, and even at times hypercritical, trial than that proposed by Kolmer.[1]

It is the purpose of this communication to summarize succinctly the reports thus far made upon this technic with a view to ascertaining the present status of the method with particular reference to (a) delicacy and sensitivity; and (b) the specificity of the reactions obtained with it.

It is impossible in the limits of the time allowed to present a full consideration of the minutiae of the various reports nor is it deemed necessary as a detailed analysis of the data to be here briefly summarized will be presented in another place.[2]

In Table I are gathered the investigations upon the analysis of which this report is based, comprising a total of 88,539 reactions conducted and reported upon by 35 different workers.

It is quite probable that no other technic has been subjected to comparative investigation in so large a series and the value and significance of the findings so obtained are enhanced by, if indeed they do not arise in essence from, the very multiplicity of the workers and the variety of methods with which the Kolmer test was compared.

The mere collection of a large number of tests is not of value per se; their significance and the value of the conclusions to be drawn from them in this instance is dependent upon the following factors and conditions forming an integral part of the investigations and their conjoint results:

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*From the Laboratories of the Atlantic City Hospital.

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TABLE I

TOTAL REACTIONS REPORTED BY WORKERS INVESTIGATING KOLMER'S TEST

AUTHOR	NUMBER OF TESTS
Cohen and Haythorn	1,400
Dutton and Thompson	501
Harper and Curtis	120
Hartman and Reyner	6,947
Hartman and Reyner	20,307
Irvine and Stern	5,162
Johns and Jones	500
Kellogg	9,307
Kellogg, Wells, and Beck	506
Kilduffe	1,014
Kilduffe	2,000
Kilduffe	104
Kilduffe	2,180
Kilduffe	300
Kolmer	355
Kolmer and Denney	159
Lorenz and Bleckwenn	997
Maynard	806
Ottenberg and Wisler	204
Palmer	2,500
Palmer and Gibb	363
Palmer	2,502
Rockwood and Sanford	500
Sanford	4,740
Butler	16,772
Goodhue	700
Schamberg and Greenbaum	3,000
Schamberg and Klauder	3,000
Shivers	320
Smith	94
Total	88,359

1. In each instance the Kolmer technic was compared with a method regarded by the authors as reliable and sensitive and thoroughly evaluated by past experience.

2. All the investigations were made and controlled under strictly experimental conditions in that every effort was made to collect full clinical data on the tested cases, particularly where there was disagreement between the methods used.

3. A sustained effort was made to subject the Kolmer technic to stringent trial in those conditions generally accepted as giving false positive reactions.

4. With the exception of Dr. Kolmer (reporting 459 reactions of the total), none of the investigators was associated with the development of the technic and, therefore, were not prebiased in its favor.

The adaptability of the Kolmer technic to and its conformity with the requirements of the laboratory and the serologist for a standard method have been fully discussed elsewhere.

As a standard method must be adapted to varied conditions from the ideal to the relatively vile, the incidence of anticomplementary reactions in the Kolmer test is of practical interest. A personal experience in over 10,000 tests shows an incidence of only 0.7 per cent and the remarkable freedom of the method from anticomplementary reactions is commented upon by all observers.

It cannot be too strongly emphasized that to obtain the excellent results possible with the Kolmer technic *it must be used exactly as described by the author to the minutest detail and that deviations, no matter how apparently minor, will be reflected in a diminution of the sensitivity and delicacy of the test.*

By reason of the long period of primary incubation the technic is not economical in time and objection has been made because of this fact.

Kolmer has stated in his original communication that while, if compulsory, a four-hour incubation may be used instead of the overnight period, the delicacy of their action is affected.

Because some may hesitate to adopt the method because of the delay in reporting, and to ascertain the difference referable to variations in the primary incubation period, I have recently studied a series of 300 serums tested by the quantitative technic but with differing incubation periods as follows:

1. Ice chest four hours at 6°-10° C.
2. Ice chest 16-20 hours at 6°-10° C.
3. Ice and brine bath four hours at 6°-10° C.

The results of the investigation, which have been reported elsewhere,[3] quite definitely demonstrated that not only was the quantitative character of the test more clearly brought out by the longer incubation period, but, what is even more important, while the number of positive reactions detected by both four-hour periods was practically the same, there were 9.6 per cent more positive reactions—mainly one and two tube fixations—detected by the overnight period.

The overnight period of primary incubation, therefore, is a valuable, integral, and important part of the technic and cannot be disregarded except at the expense of delicacy.

This being true, objections based upon this point cease to be valid.

Of particular interest are the conclusions formed as to the specificity of the Kolmer test.

Specificity implies that positive reactions shall not be obtained in conditions other than syphilis. As the complement-fixation test as applied to syphilis is not a true antigen-antibody reaction in a biologic sense, all that can be hoped for is a *relative* specificity.

The practical specificity of the reaction in syphilis, therefore, depends upon whether or not the lipotropic reagin responsible for the reaction in syphilis may be present in other conditions also. It is known that it exists in one other condition, frambesia or yaws, possibly because of the close biologic relationship existing between its cause, the Spirocheta pertenuis, and the Spirocheta pallida.

In Table II are gathered the various conditions other than syphilis in which nonspecific reactions have heretofore been obtained and which were investigated by the Kolmer test.

Large series of these conditions are neither easily nor rapidly obtained for obvious reasons; nevertheless, the total number possesses a definite significance and indicates that false positive reactions are extremely rare with the Kolmer technic *if, indeed, they occur at all.*

TABLE II

RESULTS WITH THE KOLMER TECHNIC IN NONSYPHILITIC CONDITIONS REPORTED AS GIVING FALSE FIXATIONS WITH OTHER METHODS

DISEASE	AUTHOR	NO. TESTED	FALSE POSITIVE REACTIONS
Tuberculosis, pulmonary	Hartman and Reyner 100 Kilduffe 104	204	None
Diabetes	Hartman and Reyner 86		None
	Kilduffe 3		None
	Kolmer and Steinfield 35		None
	Rockwood and Sanford 500	624	2 "probable"
Pregnancy	Hartman and Reyner 130		None
	Kilduffe 108		None
	Irvine and Stern 90		None
	Smith 94		None
	Cohen and Haythorn ??	422	1 "probable"
Pneumonia, lobar	Hartman and Reyner 45		None
	Kilduffe 14		None
	Kolmer and Steinfield 15	74	None
Uremia and Nephritis	Kilduffe 3		None
	Kolmer and Steinfield 14	17	None
Leprosy	Kolmer and Denney 159	159	None
Malaria	Kolmer 25	25	None
Pernicious anemia	Kolmer 2	2	None
Jaundice	Kolmer and Steinfield 15	15	None
Hypercholesterolemia	Thalhimer and Hogan "large number"	?	None
Thyroid disease	Kilduffe 1	1	None
Gastric carcinoma	Kilduffe 1	1	None
Scarlet fever	Kolmer "large number"	?	None
Trypanosomiasis (animal infections)	Kolmer "large number"	?	None
	Total	1,544	

The conclusion is warranted that when a positive reaction is obtained with Kolmer's test in the face of discordant or absent clinical data, the burden of proof rests upon the clinician and a thorough, exhaustive, and meticulous search for clinical evidence of syphilis is indicated.

There is a remarkable unanimity of opinion among the investigators reporting as to the freedom of the Kolmer test from false positive reactions.

Cohen and Haythorn[4] note "one probable false positive in a child with meningitis"; and "one probable false positive reaction in pregnancy"; and Rockwood and Sanford[5] report "two probable false reactions in diabetes."

An important feature of this high degree of relative specificity is the added significance imparted to weakly positive reactions, those which with other methods could only be regarded as doubtful. Unexpected and weakly positive reactions by Kolmer's method, therefore, cannot be disregarded and may be justly looked upon as due to clinically undetected syphilis.

So overwhelming is the evidence of the specificity of the Kolmer test that when clinical opinion disagrees it must be taken into consideration that clinical evidence and clinical judgment may sometimes be in error.

The delicacy of the Kolmer test is well shown in the results obtained with it in varying stages of syphilis tested under varying conditions.

The arbitrary period after the appearance of the primary lesion when the complement-fixation reaction may be expected to become positive has been set at from twenty to thirty days. It is interesting to note in view of this fact, and as indicative of the delicacy of the technic, that positive reactions—fixation occurring in more than one tube—have been reported as early as three days (Irvine and Stern), and four days (Kilduffe), after the appearance of the chancre.

FALSE NEGATIVE REACTIONS WITH KOLMER'S TEST

The fact that false negative reactions occur in relatively small numbers is an efficient rebuttal of the fear that the test might be too delicate.

The influence of natural antisheep hemolysins in any technic using an antisheep hemolytic system must always be considered in relation to the production of false negative reactions. Kolmer states their effect to be "practically negligible" in his technic. Maynard[6] using an antihen system in his routine test, notes 11 per cent of false negative reactions with the Kolmer technic, offset, however, by 6 per cent of false positive reactions with the antihen technic; and Ottenberg and Wisler[7] found 8 per cent of false negative reactions with the Kolmer method.

In view of the specific character of the positive reactions by Kolmer's method it becomes of interest to determine the influence of the natural antisheep hemolysins in human serum as a cause of false negative reactions and to determine if their prior absorption sufficiently increases the delicacy of the method to warrant this addition to the technic.

I have shown that, while the anomalous reactions occasionally occurring in the first tube of the quantitative technic may occur in hemolysin-free sera, the most frequent cause is the presence of relatively large amounts of natural antisheep hemolysin in the tested serum.[8]

I have also investigated the influence of the natural antisheep hemolysins in human serum upon the production of false negative reactions with the quantitative test.[9]

In a series of 300 sera negative to the quantitative test, the natural hemolysins were removed by absorption and the sera retested after absorption. It was found that 10 per cent of weakly and moderately positive sera originally gave false negative reactions due to their relatively high content of antisheep hemolysin.

In view of these results and also of the specificity of the positive reactions, prior absorption of the natural hemolysins may be advisable.

AGREEMENT WITH CLINICAL FINDINGS

The conformity of the Kolmer test to this clinical requirement in high degree is specifically commented upon by many workers and it is fair to state that in this respect the method again far surpasses all others.

ADVERSE REPORTS

There were no adverse reports in the series under consideration, all workers agreeing that the method was sensitive, reliable, did not give false positives and but few anticomplementary reactions and that there was a high percentage of agreement with the clinical findings.

There are only two definite criticisms: (1) The occurrence of a definite number of false negative reactions; and (2) the fact that the Kolmer method requires more time, more tubes, and a little more labor.

The first of these is valid and demands study and has already been referred to.

It is difficult to believe, however, that any serologist would seriously urge as a valid objection to the adoption of an efficient, superior and specific test the fact that it was a little more time-consuming or a little more laborious. It is much more likely and easier to believe that this objection is really an indirect expression of a reluctance to cast aside a familiar method for a new one; certainly an objection based upon such grounds would be difficult and embarrassing to uphold.

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COMPARISON OF RESULTS WITH KOLMER-WASSERMANN METHOD AND KAHN PRECIPITATION TEST*

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IN a paper[1] published some months ago we showed the results found in subjecting 2000 sera to the Kahn precipitation test, using his latest modification,[2] and to a Wassermann technic run with plain alcoholic antigens and with an 18-hour ice-box fixation period.

In that investigation we obtained an absolute check of 89.5 per cent with both tests and when the ++++, +++ and negative results were grouped together we found what we called a "diagnostic check" in 90.5 per cent of the cases.

There were 105 cases where one method showed a reaction of diagnostic strength (++++ or +++) while the other was of lesser degree and these cases were grouped as follows:

Wassermann ++++ or +++,	Kahn 0	10 (0.5 %)
Wassermann ++++ or +++,	Kahn +	19 (0.95%)
Wassermann ++++ or +++,	Kahn ++	30 (1.5 %)
Wassermann more strongly positive than Kahn		59 (2.95%)
Kahn ++++ or +++,	Wassermann 0	16 (0.8 %)
Kahn ++++ or +++,	Wassermann +	13 (0.65%)
Kahn ++++ or +++,	Wassermann ++	17 (0.85%)
Kahn more strongly positive than Wassermann		46 (2.3 %)

Nine of the 10 Wassermann-positive, Kahn-negative cases gave definite histories of syphilis with treatment while among the 16 Kahn-positive, Wassermann-negative cases, 12 were definitely syphilitic; with 3 we were unable to obtain any data and 1 was probably a false positive.

Having convinced ourselves of the superiority of the Kolmer method over our former technic we adopted it as our standard and the present series of 1600 cases was tested by the Kolmer method in comparison with the Kahn.

In order to establish some basis of comparison in reporting the results with the two tests we gave a value of 0 to 4 to the reaction obtained in each tube of the respective sets (3 tubes in Kahn and 5 in the Kolmer), and then arbitrarily assigned the following values to the sum total of the results in all the tubes of each set.

+	= Total of 1 to 2 with Kolmer	= Total of 2 to 4 with Kahn
++	= Total of 3 to 6 with Kolmer	= Total of 5 to 7 with Kahn
+++	= Total of 7 to 10 with Kolmer	= Total of 8 to 10 with Kahn
++++	= Total of 11 to 20 with Kolmer	= Total of 11 to 12 with Kahn

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For example, a Kolmer reading of 4—3—1—0—0 giving a total of 8 units we called +++, while a Kahn reading of 2—3—4, we also graded as +++.

We followed the exact details of the Kolmer method, except that we added the salt to the antigen in making our antigen dilution, while the only variation from the Kahn was in using a 15-minute inactivation period instead of 30, and in shaking the serum antigen mixture for four minutes by hand instead of for two minutes in a shaking machine, as we have not found that the longer inactivation period made any difference and Kahn himself advocates a two to four minute shaking period.

In this series of 1600 cases we got an absolute check in 1471 or 91.9 per cent, and by grouping the ++++, +++ and negative reactions together got what we called a "diagnostic check" in 1501 or 93.8 per cent.

There were 59 cases showing considerable divergence in results, ranging from negative with one test to ++++ with the other, practically all being treated cases.

These cases showed the following:

Wassermann ++++ or +++,	Kahn 0	5 (0.31%)
Wassermann ++++ or +++,	Kahn +	11 (0.69%)
Wassermann ++++ or +++,	Kahn ++	11 (0.69%)
Wassermann stronger than Kahn		27 (1.68%)
Kahn ++++ or +++,	Wassermann 0	18 (1.1 %)
Kahn ++++ or +++,	Wassermann +	3 (0.19%)
Kahn ++++ or +++,	Wassermann ++	11 (0.69%)
Kahn stronger than Wassermann		32 (2.0 %)

The great significance of our findings lies in the fact that there were five cases frankly negative with Kahn and strongly positive with the Wassermann and 18 where the opposite result occurred. Had dependence been placed on only the one method a certain percentage of positive results would have been missed.

The Wassermann-positive, Kahn-negative cases were:

1. 1478—old latent lues—last treatment 5 years ago.
2. 1465—chancre 5 weeks duration.
3. 1454—multiple gumma of leg.
4. 1999—old treated case—has severe diabetes.
5. 2071—latent lues—treated 2 years ago.

The Kahn-positive, Wassermann-negative cases were:

1. 969—last treatment 7 weeks ago.
2. 740—last treatment 8 days ago.
3. 1171—last treatment 1 month ago.
4. 1026—last treatment 3 months ago.
5. 1460—last treatment few months ago.
6. 1821—last treatment few months ago.
7. 1573—old treated case.
8. 2193—old treated case.
9. 2314—old treatment case.
10. 979—old treated case.
11. 1723—chancre 7 to 10 days.
12. 1147—child. No history or symptoms.
13. 1587—no history or symptoms. Severe diabetes.
14. 1613—colored. No history or symptoms.
15. 1554—history not known.
16. 1181—history not known.
17. 1427—physician recovering from glandular fever. No history or symptoms.
18. 1625—physician's wife. Has early P. A. No history or symptoms.

These 18 cases comprise 6 recently treated, 4 old treated cases, 1 early chancre, 3 with no definite history of symptoms as determined by the attending physician, 1 with no available data and 2 where syphilis could presumably be excluded.

TABLE I

SUMMARY OF 1600 KAHN AND KOLMER TESTS

	WASSERMANN				
	++++	+++	++	+	0
KAHN					
+ + + +	223	29	10	2	10
+ + +	7	11	1	1	8
+ +	7	4	5	1	11
+	7	4	5	1	14
0	2	3	1	2	1231

We still find that a not inconsiderable number of sera give weak precipitates at times, consequently we do not attach much importance to ± or + reactions, but where frankly positive results are obtained we feel that the patient should be subjected to further study.

The simplicity of the test and the rapidity with which the reactions may be read make it an ideal check on the Wassermann method, and we feel a much greater confidence in the correctness of our results where a serum is subjected to both tests than we formerly had in depending on the Wassermann set up alone.

The antigen-salt mixture, however, is an extremely unstable body and careful controls are just as essential as they are in the fixation method.

SUMMARY

Examining 1600 sera we obtained a practical check in 93.8 per cent of the cases.

The divergence between the two tests lies almost wholly among the treated cases.

The Kahn precipitation test furnishes an ideal check for the Wassermann reaction and will "pick up" a certain small percentage of positive results which may be missed by the older method. On the other hand, certain cases may show a positive Wassermann reaction and a negative Kahn.

To obtain the greatest accuracy possible all sera should be subjected to both the Kahn and Wassermann methods and where the results differ radically further study of the case is indicated.

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THE KAHN PRECIPITIN TEST AS COMPARED WITH THE KOLMER COMPLEMENT-FIXATION TEST*

By Alfred S. Giordano, M.D., M.Sc., South Bend, Ind.†

SINCE Kahn, in 1922, proposed his flocculation reaction for syphilis, there have appeared numerous reports supporting his contention that his test compares very well with the Wassermann test. The present report is based on a comparative study of 2540 consecutive sera tested with Kolmer's quantitative system and Kahn's latest modification of his own precipitin reaction. Forty of these are not included in this report since full clinical data was not obtainable. The sera were from patients admitted to the St. Joseph Hospital, the Epworth Hospital and the United States Public Health Clinic. A very large percentage of this material is made up of sera obtained from patients under treatment for syphilis, of whom a complete record of the progress following treatment at various intervals is available.

The technic employed for the Wassermann test is the standardized quantitative complement-fixation test described by Kolmer. This test was followed in every detail with the single exception, that the sera were inactivated for 20 minutes instead of 15 minutes. The precipitin reaction used is that described by Kahn, in which the ether extraction for the antigen is carried out at room temperature by four consecutive washings of the powdered muscle at ten-minute intervals with 100, 75, 75, 75 c.c. of ether respectively. The subsequent alcoholic extraction is carried out for three days at room temperature. Various amounts of stock alcoholic extract, sufficient to last about a month, were fortified by the addition of 6 mg. of cholesterin per c.c. The unit of each batch of cholesterinized antigen was determined by titration and 0.05 c.c. and 0.025 c.c. amounts were used in the test. These quantities were found sufficiently satisfactory and accurate for routine work, instead of using 0.05 c.c., 0.025 c.c. and 0.0125 c.c. as advocated by Kahn. The readings were made after overnight incubation at 37° C., after adding 0.5 c.c. of normal saline. It must be emphasized, in this connection, that overnight incubation is not necessary. The original technic was adhered to in order to complete the series herein reported. A second series already in progress using 15 minutes water-bath incubation is proving to be even superior to overnight incubation. The scale used is as follows:

Precipitate with one or more large clumps = 4+
A large flocculent precipitate = 3+
Medium size flocculi = 2+
Fine granules visible to the naked eye and not soluble by shaking = 1+
No perceptible precipitate negative.

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When referring to the Kolmer quantitative fixation test, the term weak-positive includes all reaction showing 50 per cent or less complement fixation in the first two tubes. Strongly positive includes all reactions showing complement fixation of more than 50 per cent in two or more tubes. In the Kahn test 1+ and 2+ are considered weakly positive and 3+ and 4+ strongly positive. This terminology, of course, is not that described by either Kolmer or Kahn and I have used it because of its brevity.

Tables I, II and III represent a summary of the results obtained.

TABLE I

Kolmer strongly positive and Kahn strongly positive	1685 or 67.4%
Kolmer negative and Kahn negative	540 or 21.6%
Kolmer weakly positive and Kahn weakly positive	92 or 3.68%
Total agreement in type and degree of reaction	2317 or 92.68%

TABLE II

Kolmer strongly positive and Kahn weakly positive	53 or 2.12%
Kolmer weakly positive and Kahn strongly positive	28 or 1.12%
Total agreement in type but disagreement in degree of reaction	81 or 3.24%

TABLE III

Kolmer strongly positive and Kahn negative	21 or 0.84%
Kolmer weakly positive and Kahn negative	27 or 1.08%
Kahn strongly positive and Kolmer negative	22 or 0.88%
Kahn weakly positive and Kolmer negative	32 or 1.28%
Total disagreement in reaction	102 or 4.08%

Table I shows that 2317, or 92.68 per cent, of the sera reacted in complete agreement both in type and degree of reaction, while 81 or 3.24 per cent vary only in intensity of reaction as shown in Table II. This phase is very interesting and I have noted that it occurred in my series only in patients who had received treatment. These sera, when rechecked, gave practically always identical results, thus excluding technical error. Rochstraw and Malcom state that this disagreement in degree of reaction never occurred in their experience. However, the total per cent of agreement closely checks with them.

Table III represents a group of 102, or 4.08 per cent, of the sera in which there is a complete disagreement. The 21 sera that were strongly positive with Kolmer and negative with Kahn, were from patients under treatment for lues. Fourteen of these yielded similar results on rechecking, while the other seven could not be repeated as the patients were ambulatory and their whereabouts unknown. Of the 27 sera in which the Kolmer test was weakly positive and Kahn negative, 20 were from patients under treatment from one to two years, 5 had a history of primary lesion ten years or more before and had received very little treatment, and two had no history or clinical evidence of syphilis. The reaction in these last two cases was 2, 1, and 2, 2, respectively. Of the 22 strongly positive Kahn and negative Kolmer, twenty were from patients under treatment for lues, one had a history of a primary infection fifteen years before with insufficient treatment and symptoms of tabes dorsalis, while

one had no clinical evidence of syphilis, although there was a history of exposure. Finally, of the 32 weakly positive Kahn and negative Kolmer, ten had been under treatment for syphilis and discharged, nine were still under treatment and thirteen had no history or clinical evidence of syphilis.

In connection with these thirteen apparently false weakly positive sera, it must be emphasized that I have used overnight incubation which, while it renders the precipitates more conspicuous, does, however, increase the possibility of error.

SUMMARY

In summing up, it is evident that the two tests run parallel in about 96 per cent of the sera. Both tests occasionally render a false negative which is usually picked up by one or the other when the two tests are run parallel. When we consider that antigens vary in polyvalance, this discrepancy is to be expected by any two tests employing different antigens. The Kahn test, however, has several points of advantage, namely, it excludes the hemolytic system which does away with the necessity of expensive equipment, so that the test is available to small institutions maintaining a moderately equipped laboratory; anticomplementary reactions are eliminated and may in the future solve the problems associated with this phenomenon; and lastly, it can be utilized to a great advantage in testing donors for emergency transfusions. One disadvantage of the Kahn test, in my experience, has been the reading of the weakly positive sera. These are often difficult to interpret and on that account I have never felt secure in reporting a weakly positive Kahn unless I could check it with a complement-fixation test. On the whole, I believe that the Kahn test is a valuable addition to the diagnostic laboratory. It is an excellent check on the complement-fixation test because of its accuracy, simplicity in technic, rapidity in performance and inexpensiveness.

CLINICAL STUDY OF KAHN PRECIPITATION TEST AND KOLMER COMPLEMENT-FIXATION TEST*

By Robert Lee Kelly, M.D., Philadelphia, Pa.

WASSERMANN, Neisser and Bruck,[1] introduced to the medical world the complement-fixation test for syphilis in 1906, and since that time it has undergone several modifications in the hope of making it more practicable and more sensitive with greater specificity; but in all of its modifications the fundamentals of the test are the same. The disadvantages of the complement-fixation test are well known. The number of biologic reagents that enter into the test increase the source of error. The most important ingredient, complement, is

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not very stable, and if it should deteriorate without restandardization, one might get false reactions. Although this is overcome by the use of proper controls, many sera contain natural antisheep hemolysin which, if not absorbed, may give false negative reactions. Even if all the reagents used were perfectly stable, the many exact manipulations necessary in making the test make it far from foolproof, especially in the hands of the inexperienced worker.

For the last few years many workers have been trying to develop a biologic test for syphilis which would equal or excel the complement-fixation reaction in sensitivity, but which would be devoid of its technical difficulties and errors.

The visible precipitation and flocculation of a protein solution by a specific antibody has long been known under the name of precipitin reaction. Michaelis[2] in 1907 added a syphilitic alcoholic extract antigen to syphilitic serum and noted flocculation, and the absence of the same in nonsyphilitic serum. From his observation he drew the conclusion that the flocculation noted in his experiment was due to a specific precipitin reaction. Later, other investigators, using as antigen extracts from nonsyphilitic organs, observed the same colloidal precipitation reactions with syphilitic sera, and the absence of precipitation in nonsyphilitic sera or normal sera. There have been several tests devised based upon this phenomenon. The most important precipitation tests are those of Meinicke, 1917, followed by Sachs-Georgi, 1918, Dreyer-Ward and Kahn, 1922. I will not go into detail with the technic employed in the first three, as I am only interested in the Kahn modification. Within the last two or three years there has been much in the literature on Kahn's precipitation test. Most of these investigators have compared the Kahn precipitation test with the Wassermann reaction or one of its modifications, and the results obtained have checked within from 82 per cent to 100 per cent. The variations are probably due to the different modifications of the Wassermann reaction used, and there will not be uniformity of results until it is checked with a standardized complement-fixation test. It is this that I have attempted to do. The two most important questions with any serologic test for syphilis are specificity and sensitiveness,[3] all other factors of the test should be of secondary importance. The practitioner is not so much interested in the serologic test in frank and extensive manifestation of syphilis, for in those cases it is not necessary for serum reaction to establish the diagnosis; but in those cases which have atypical symptoms and indefinite clinical findings, the serologic test is of importance, and the test must be delicate and at the same time possess practical specificity and be free from nonspecific reactions.

In my series of cases I had an ideal opportunity to study clinically the Kahn precipitation test in comparison with Kolmer's new quantitative complement-fixation test for syphilis.[4] Kolmer claims for his test increased sensitivity and practical specificity, and reports from other investigators corroborate his statement. Palmer and Gibb[5] examined 329 patients, using the older method and Kolmer's technic and found that the latter shows greater delicacy and specificity and fewer anticomplementary reactions. Kilduffe[6] examined 1014 sera with Kolmer's technic and the old routine method and did not find any false positive reactions. He reports that the Kolmer technic

possesses greater sensitivity, flexibility and elasticity. Schamberg and Greenbaum[7] after conducting more than 4000 tests upon 400 cases, did not find a false positive reaction. F. W. Hartman and C. E. Reyner[8] used the technic in over 6000 cases and report the method as a "specific test for syphilis." These writers[9] in a recent communication report 20,000 tests performed by Kolmer's method and with no false positives, and in their review of the literature of 30,000 tests have encountered no false positives. They claim the test is ideal so far as absence of false positive results are concerned.

There has not been much investigation done on the question of the specificity of the Kahn precipitation reaction for syphilis, most workers performing the test in parallel with one of the older modifications of the Wassermann reaction. Keim and Wile[10] were the first investigators to study the clinical application of the test, and report that it compares favorably in sensitivity with the standard Wassermann reaction. Other investigators have corroborated their findings. Moody[11] in 1500 tests found agreement in 98 per cent. Detweiler[12] in 1540 sera reports agreement in 94.2 per cent and says that it is almost as reliable as the Wassermann reaction. Holmes[13] in 1000 cases noted a close correlation between the two tests, with the balance in favor of Kahn. Letterer[14] in 1000 cases found agreement surprisingly accurate, but thinks that the test should not supplement the Wassermann test. Young[15] examined 5080 sera and found agreement in 93.03 per cent, relative agreement in 5.96 per cent and complete disagreement in 1.01 per cent. Levine[16] in comparative tests found that of 258 sera there was agreement in 86.4 per cent, but says the Kahn test should not replace the Wassermann, but should be used as a check. Dulaney[17] reports 900 cases in which the test compared in about 88 per cent, and says that it should be used as a check on the Wassermann reaction. Ide and Smith[18] examined 2165 sera and found the results identical when more than two plus positives are counted. Strumia[19] in comparative tests with 624 sera found that the Kahn test agreed with Kolmer's complement-fixation test in 83.4 per cent of sera. Keim and Kahn[20] in a recent communication reported a study of 2600 cases in comparison with two Wassermann tests—one highly sensitive and the other more conservative in character—reported the following conclusions: In 25 primary cases of syphilis the Kahn test was found more sensitive than either of the two Wassermann tests. In 64 secondary cases there was agreement in all three methods. In 87 tertiary cases, excluding the cerebrospinal type, the Kahn test was found more sensitive than either of the Wassermanns. In 192 cases of cerebrospinal involvement Kahn falls midway between the two Wassermann tests. In 218 cases of latent syphilis Kahn's test compares favorably with the reaction of the long ice box fixation. In 39 cases of congenital syphilis it was more sensitive than either of the two Wassermann tests. In 1975 nonsyphilitic controls Kahn gave two one-plus and two two-plus reactions in dermatologic conditions apparently nonsyphilitic.

Inasmuch as most of the previous studies of the value of the precipitation tests have been in comparison with the older nonstandardized Wassermann tests, it seemed to me that it would be desirable to compare a series of cases, using Kahn's latest modification of the precipitation reaction, with tests on the same sera by the most exacting of the modern modification of the Wassermann,

namely, Kolmer's standardized technic, which has been repeatedly shown to be practically specific. The sera obtained were tested by Miss Yagle in Dr. Kolmer's Laboratory, using his technic; and a portion of each serum was tested by me independently, using the Kahn technic. In order to be unbiased in my opinion, I read and recorded my findings before knowing the results of the tests performed by Miss Yagle.

EQUIPMENT

Glassware.—The test tubes for making the standard antigen were of standard size, 5.5 cm. in length and 1.5 cm. in width. The tubes for conducting the test were 7.5 cm. in length and 1 cm. in diameter. All test tubes were perfectly clean and free from acid and alkali and sterilized in hot air sterilizer. The pipettes were chemically clean and sterile. Five c.c. pipettes graduated to 0.1 c.c., one c.c. pipettes graduated to 0.01 c.c. and calibrated to the tip, and 0.2 c.c. graduated to 0.001 c.c. quantities were used.

Antigen.—The antigen used in my series of tests was furnished by Dr. Kahn, ready for dilution and sent to me at about one month intervals. The titration was usually 1 c.c. standard antigen plus 1 c.c., 1.1 c.c. or 1.2 c.c. physiologic salt solution.

Physiologic Salt Solution.—This was prepared by adding 8.5 grams of chemically pure sodium chlorid in 1000 c.c. of freshly distilled water in a chemically clean and dry flask, filtering and sterilizing in an Arnold sterilizer for one hour.

Technic.—The technic employed in conducting the tests was that advised by Kahn, with one exception. In performing his tests the sera were inactivated for thirty minutes at 56°. Kolmer[21] in his modification of the complement-fixation test found that heating the serum for thirty minutes at 56° results in the destruction of a portion of syphilis "reagin," and that heating for fifteen minutes at 55° is sufficient and results in less destruction of "reagin." As my series of cases were compared with Kolmer's modification of the complement-fixation test, his method of inactivation of sera was carried out; the tests were inactivated for fifteen minutes at 55° and were then put in the water-bath for fifteen minutes before adding 0.5 of physiologic salt solution.

Serum.—Specimens of blood were drawn and allowed to stand overnight. The following morning the sera were separated from the clots and made free from red cells, fibrin and other particles which would interfere with accurate readings. They were prepared as for the complement-fixation test (Kolmer) and inactivated for fifteen minutes at 55° C. as above noted. Each serum was then divided, a part being used for the complement-fixation test and a part for the precipitation test.

Antigen Dilution.—One c.c. of standard antigen was placed in a standard mixing tube, and approximately the same amount of physiologic salt solution as determined by titration was placed in a second standard mixing tube. The salt solution was quickly poured into the antigen, and without waiting for it to drain it was poured back and forth for six or eight times to insure thorough mixing. This antigen was allowed to stand for a few minutes before using, but was used in test within a half hour.

Conducting of the Test.—Three test tubes were employed for each serum tested. In the first tube was placed 0.05 c.c. antigen dilution, in the second tube 0.025 c.c., and in the third 0.0125 c.c. The antigen dilution was delivered to the bottom of each tube. Then to the antigen in each tube was added 0.15 c.c. of serum to be tested. After the serum was added, the test was shaken for two or three minutes to insure thorough mixing, and incubated in the water-bath at 38° for fifteen minutes. Five-tenths c.c. of physiologic salt solution was then added and the reading made. This was done before a window with dark background. The strongly positives were easily read, the weakly positives and doubtful reactions were read by slanting the tubes and looking for precipitation in the thin layer of fluid. After this reading was made the test was placed in the ice box overnight, and final reading made the following morning. Ice box incubation increased the degree of precipitation in the weaker reactions.

Method of Control.—Each test had the following controls: (1) Antigen. (2) Known positive serum. (3) Known negative serum. Each control contained the same amount of antigen dilution and serum as the test, while the antigen contained only the normal salt solution.

TABULATION OF CASES

As all of my series of cases except four were old untreated cases, I have tabulated the results merely as positive and negative, instead of giving the degree of positivity. In this type of case, as we are not using the test as a guide for treatment, we are not as much interested to know the degree of positivity of serum reaction.

LATENT SYPHILIS

NO. OF SERA	KAHN TEST	COMPLEMENT FIXATION	AGREEMENT	DIVERGENCE
67	66 positive	64 positive	63	4

Divergence—4 cases

Case No.	Kahn Test	Complement Fixation
1. (C.W.)	Positive	Negative
2. (J.S.)	Positive	Negative
3. (J.K.)	Positive	Negative
4. (G.D.)	Negative	Positive

The histories and clinical findings of these latent cases were rather indefinite, the majority of them being referred from other departments to Schamberg's Clinic for complement-fixation test. A few gave a history of a primary lesion; others denied all knowledge of infection. In the series of 67 cases there was disagreement in four cases. Three gave a positive Kahn test and a negative complement-fixation test, and one gave a negative Kahn and a positive complement-fixation test. It will be of interest to note that five cases out of sixty-seven cases of latent syphilis gave a negative complement-fixation test until the natural antisheep hemolysin was absorbed. After this they were positive. Also one case gave a positive Kahn and a negative complement-fixation test, but after four weeks of treatment the complement-fixation test was strongly positive. There is the possibility of missing a small percentage of positive cases unless the antisheep hemolysin is absorbed routinely.

Case Histories and Clinical Findings in four cases showing divergence of precipitin and complement-fixation tests follow:

CASE 1.—C. W., a man, aged fifty-four years, came to the Clinic October 28, 1918, with the following history: "Had a primary lesion two and one-half years ago, duration two or three months. Rash appeared and has been appearing and disappearing for the last two years. Has sore mouth, tongue beefy red, glazed, shows milky patches and is slightly lobulated. His wife has had three children, all of whom are living. She had one miscarriage eighteen years previously." The blood was taken and the Wassermann found to be strongly positive. The patient received ten weekly injections of arsenobenzol over a period of two months, with the Wassermann, showing strongly positive. The patient disappeared from the Clinic at the end of this time and did not report until January, 1925. Two blood tests taken then which were positive to the Kahn test and negative to the complement-fixation test.

Physical Examination at this time showed the following: Glossitis and lingual sclerosis; enlargement of the liver with nodular edges. The Department of Neurology reported the following: Pupils regular, react slightly to light and in accommodation; somewhat myotic, ocular movements normal; cranial nerves normal, reflexes all normal. Their conclusion is "The symptoms of neurosyphilis not very definite. Pupils are rather rigid. Slight tremor of facial muscles, failure of memory and fatigability. Might indicate a pre-paretic state. Whether or not I think would depend somewhat on serological test."

Spinal fluid examination showed the following: Complement-fixation test, negative, colloidal gold curve 1123430000, cells—17 per c.mm., globulin not increased.

CASE 2.—J. S., a man, aged thirty-nine years, came to the Medical Clinic January 1, 1925, complaining of a "heavy feeling" in the epigastric region. Physical findings: No palpable lymph nodes; bone and joint findings normal; patellar reflex active; pupils unequal and irregular, reaction to light sluggish, to accommodation normal; mouth and pharynx negative; lungs negative; heart rhythm irregular with dropped beat, systolic murmur at apex; liver normal.

The patient's wife is living and well. Three children are living and well. The last pregnancy resulted in a miscarriage at four months. The patient gave a history of being treated for syphilis three years ago by his private physician, and was told that he did not need any more treatment. His diagnosis in the Medical Clinic was "chronic myocarditis." He was referred to Dr. Schamberg's Clinic for a complement-fixation test. Two tests were made at about two week intervals and both were negative. Two Kahn tests were made on the same sera and both were positive. The patient left the city before a spinal puncture could be made. However, the history of treatment for syphilis, together with the unequal and irregular pupils and sluggish reaction to light, are very suggestive.

CASE 3.—J. K., private patient of Dr. Jay F. Schamberg. The patient, a man aged forty years, was first seen in December, 1912, with a history of having had a syphilitic infection three years before. He had a negative Wassermann at the time of his first visit to Dr. Schamberg, but owing to the inadequacy of his earlier treatment, he was given a course of arsphenamin injections. The patient resided in another city and his visits were rather irregular. Although free of symptoms, his Wassermann was positive in March, 1913. Another course of arsenical injections was given, followed by mercury. Two months later the Wassermann was still positive. Mercurial injections were then administered. In September, 1913, a quantitative test gave a four-plus reaction with four different antigens. Further treatment was given and in April, 1914, it was three-plus. Four months later it was two-plus. In January, 1915, the complement-fixation test was as follows: Cholesterinized beef heart four-plus, alcohol extract syphilitic liver four-plus. The patient was further treated, but at irregular intervals. Nine years then elapsed before the next visit in December, 1924, when the patient came to request an opinion as to eligibility for marriage. He had been free of all symptoms for over ten years and was in excellent health. His Wassermann by the Kolmer method was completely negative, but it was one-plus positive by the Kahn test. Owing to this, a second Kolmer test was performed two weeks later,

with again a negative outcome. Spinal puncture was performed and the four usual tests made, which were all negative. Permission for marriage was given.

It is impossible in this case to state whether the Kahn test was more sensitive than the Kolmer, or whether it gave a false positive.

CASE 4, G. D., a woman, aged thirty-four years, had a blood test taken by her private physician and this was found to be positive. She was referred to Dr. Schamberg's Clinic for antisyphilitic treatment. On examination nothing of importance was found. A complement-fixation test on the blood serum was positive, and a Kahn test on the same serum was negative. The complement-fixation test and the Kahn test were done on four consecutive days, with the complement-fixation test positive and the Kahn test negative. After three months treatment with neoarsphenamin another Kahn test was performed, again with negative result. The complement-fixation test during the three months treatment has been positive.

No theory is offered as to the reason for the negative Kahn test in this case.

TERTIARY SYPHILIS

NO. OF SERA	KAHN TEST	KOLMER COMPLEMENT FIXATION TEST	AGREEMENT	DIVERGENCE
34	34 Positive	34 Positive	34	0

NEUROSYPHILIS

NO. OF SERA	KAHN TEST	KOLMER COMPLEMENT FIXATION TEST	AGREEMENT	DIVERGENCE
6	6 Positive	5 Positive	5	1

Divergence 1 case

CASE NO.	KAHN TEST	KOLMER COMPLEMENT FIXATION TEST
Case 5. (M.M.)	Positive	Negative

CASE 5.—M. M., a man, aged fifty-one years, came to the Medical Clinic complaining of stiffness of the joints and aches and pains all over the body for the last six months. He has vertigo and staggering at times. On physical examination there are no palpable lymph nodes, the bones and joints are normal, the patellar reflexes are present when reinforced.

Eyes.—The right pupil is dilated, due to an injury. The left eye will not react to light or in accommodation. The heart and blood vessels are normal.

The patient was referred to Dr. Schamberg's Clinic for a complement-fixation test. The complement-fixation test was negative, and the Kahn test positive. Both tests were repeated one week later with the same results. The antisheep hemolysin was absorbed and the complement-fixation test repeated, again with negative results.

Spinal puncture was done and the following tests made and recorded: Complement-fixation test, strongly positive; colloidal gold gave a luetic curve, cell 75, and increased protein.

In this case the Kahn test appeared to be more sensitive than the Kolmer test. This case and the preceding one illustrate the importance of making both tests.

CONGENITAL SYPHILIS

NO. OF SERA	KAHN TEST	KOLMER COMPLEMENT FIXATION TEST	AGREEMENT	DIVERGENCE
5	5 positive	5 posiitve	5	0

SUMMARY

In the clinical study of 110 cases a Kahn precipitation test and a Kolmer complement-fixation test were run on the same sera. The precipitation test was done by the method advised by Kahn, with the exceptions that inactivation was

carried on only fifteen minutes, and that the readings were checked the following morning after ice box incubation. In this way it was found that the doubtful and weaker reactions became more distinct.

In tests done on 110 cases, a remarkable concordance of results was found in 105, or 95.45 per cent. This indicates a high degree of sensitivity and specificity in both tests. In the five cases that disagreed, four are known to have had syphilis and the other case is clinically suggestive. Three of the cases that are known to have been syphilitic gave a positive Kahn and a negative complement-fixation test with blood sera. One case gave a repeated negative Kahn test in the face of a persistently positive complement-fixation test. Both tests were repeated after three months of neoarsphenamine treatment, and the same discrepancy again noted. No explanation is offered for this divergence, and it will remain for future workers to ascertain whether such false negatives occur with the Kahn test. The case which gave a history of treatment and presented suggestive clinical findings of syphilis gave two positive Kahn tests and two negative complement-fixation tests on two different sera. Out of 110 cases studied, it was found that five gave negative complement-fixation tests until the natural antisheep hemolysin was removed. One case gave a positive Kahn and a negative complement-fixation test and after four weeks of neoarsphenamine treatment the complement-fixation test was reversed to strongly positive.

In this series of cases there is no convincing evidence that a positive occurred with the Kahn or the Kolmer test which was inconsistent with clinical or other serologic findings. In Case 3 no final judgment is possible as to the positive Kahn test.

The present study shows a remarkable degree of harmony between the outcome of the Kahn precipitation test and the Kolmer complement-fixation test, and indicates the high degree of sensitivity and specificity common to both. It may not be essential to carry out both tests routinely, but in doubtful cases both should be performed as they shed a useful complementary light upon each other.

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THE NEW MODIFICATION OF THE MEINICKE TEST*

By Dr. Anny Petersen Saunders, Chicago, Ill.

SINCE the introduction of the Wassermann test and especially during the World War, many laboratory workers have tried to find a serodiagnosis test for syphilis, which will combine a specific reaction with simple working methods that will not require several unstable and expensive reagents. The nature of the Wassermann test was originally explained by Ehrlich's side chain theory until it became known that antigens prepared from normal extracts gave practically the same result as those made up from syphilitic livers, which were difficult to obtain for the ever-increasing demands. After that research took a different direction. Many new theories sprang up, numerous modifications of the Wassermann test were employed, new tests recommended, of which most of them soon fell into disuse. In recent years a new kind of test, the floccule formation test, has become known, has been tried by many and has stood the proof of time. At the meeting of the "League of Nations" in Switzerland in 1924, the floccule formation test, of which the Sachs-Georgi and the Meinicke are the best known, was cited as a reliable test in syphilis, giving the same reaction as the Wassermann and being especially useful where the latter is indefinite.

Many authors have endorsed the floccule formation test. A. L. Urquart in the Lancet recommends the Sachs-Georgi test on account of its reliability and simplicity. Levinson and Petersen have found the Sachs-Georgi test to be even more sensitive than the Wassermann in certain cases of neurosyphilis. Much has been written with regard to the true nature of the Wassermann, floccule formation and other immunity tests. Kolmer in his article in "Infection, Immunity and Serum Therapy," 1923, p. 523, expressed his belief that the Wassermann and the floccule formation tests have the same fundamental mechanism. In the Wassermann test there is also the formation of floccules which have been demonstrated by means of the ultramicroscope. Both kinds of tests depend upon a specific antibody, just like agglutination and precipitin reactions. Wassermann to the last maintained that a true antibody is responsible for the complement fixation in syphilis. Meinicke explains the floccule formation by a

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special affinity of syphilitic sera for lipoidal extracts; this may be a true chemical change or an absorption. He considers the floccule formation to be a true immunity reaction, as the complement-fixation, agglutination and precipitin tests. Epstein and Paul consider the Wassermann and Meinicke reactions to be due to a specific protective colloid, which preserves the stability of the globulin molecule in normal sera and is absent in syphilis. H. G. Wells in his recently published book, "The Chemical Aspects of Immunity," expresses the opinion that at present we can say nothing more definite than that the proportion and character of serum globulins and lipoidal colloid suspensions are altered in syphilis in some unknown way, so that their stability is greatly reduced and hence there is a tendency to flocculation and to absorption of other colloids, including among them the serum colloids, which exhibit the function of complement. There is no convincing evidence that a true antibody produces the change in the behavior of the globulins.

Although the true nature of the floccule formation tests is not as yet fully understood, they doubtless are of great value. Several months ago we used a series of 500 specimens to check up on the Sachs-Georgi test in connection with the Wassermann. We proceeded to make up alcoholic extracts of dried beef heart muscle fortified with cholesterin, according to the Sachs-Georgi directions. Each new batch of antigen was titrated, using increasing amounts of cholesterin with several known positive and a few negative specimens until the proper amount for the most definite floccule formation was ascertained. The amount of cholesterin needed in the various new antigens was not at all constant, some extracts requiring more, others less for satisfactory results. A few antigens were found to be entirely ineffective and had to be discarded. All of the other antigens were rather unstable. For the test proper we mixed the inactivated blood sera with the extract, incubated for two hours and then made a preliminary reading. After that they were put back into the incubator until the final reading the next morning. The length of time required for the test proved to be the greatest difficulty in our work. Practically all of our specimens come from a distance and are from two to four days old when they reach us. Although we used a careful aseptic technic we got cloudiness in some specimens, which was due to bacterial growth. The results often were so indefinite as to make the reading very difficult.

In the fall of 1924 the author had a chance to become well acquainted with the Meinicke test, which is being used extensively in European laboratories. The Meinicke test is based upon the same principle as the Sachs-Georgi, the Kahn and other floccule formation tests, but in its present modification it is a step forward so far as simplicity in technic and a more definite reading are concerned. Meinicke uses horse heart muscle extract, without cholesterin, but with the addition of tulol-balsam. Most laboratories find it convenient to obtain the prepared extract from the Meinicke laboratories in Hagen-Westfalen, Germany, direct, since it is rather inexpensive, stable, and difficult to prepare. In the new modification test it is not necessary to wait for the formation of floccules since the production of an opacity in positive specimens is always the forerunner of the floccules and can be seen after keeping the test tubes at room temperature for one hour. The main advantage of the Meinicke test is that

specimens need not be inactivated, nor incubated and that it is completed within one hour, when a distinct reading can be made.

TECHNIC OF TEST FOR TEN SPECIMENS

Twenty small test tubes of about 4 c.c. capacity are set up in a double row. The front row is for control, the back row for the test proper, so that for each specimen there are two tubes. Of every specimen 0.2 c.c. of blood serum entirely free of cells is measured into the front and the same amount into the corresponding back row tube. All ten front row tubes receive one drop of pure formalin.

Dilution of extract: 1 c.c. of the Meinicke extract is measured into each of two 20 c.c. test tubes, which must be perfectly dry. Ten c.c. of a 3 per cent salt solution is pipetted into each of two other test tubes of even size. All four tubes are kept in a water-bath of a temperature not exceeding 45 and not falling below 40 degrees centigrade, for ten minutes. The warm salt solution is mixed as quickly as physically possible with the warmed extract, at once drawn up into a 10 c.c. pipette and 1 c.c. of the dilution added to each tube in the front and in the back rows. It is very important to mix quickly, as the suspension progressively becomes opaque, interfering with the reading later on. The control tubes in the front row and the negative tubes in the back row are entirely transparent, even though they have a slight milky appearance. The positive tubes have become opaque. This opaqueness can ONLY be seen by holding the tube up to the window in daylight. The meshes of the window screen or the wood work look hazy or cannot be seen at all when looking straight through the fluid of the tube. Out of 1000 blood serum specimens which we tested by both the Wassermann and Meinicke methods, we got the following results:

Wassermann		*Meinicke*	
negative	811	negative	811
positive	114	positive	114
anticompl.	9	negative	9
antic.	13	positive	13
doubtful	12	positive	12
doubtful	7	negative	7
negative	11	doubtful	11
positive	8	doubtful	8
negative	9	positive	9
positive	6	negative	6

The above Meinicke findings were read some two hours before the Wassermann results were known.

The Meinicke test is especially valuable in cases where the Wassermann is anticomplementary or doubtful. In our laboratory where most of the specimens are sent in from a distance we have found the Meinicke a great help when a specimen had become slightly hemolytic or anticomplementary during shipment. In those few specimens where Wassermann was distinctly contradictory to the Meinicke, we have asked for additional specimens. From our experience we can recommend the Meinicke test as a check on the complement-fixation test; it is simple, requires no special apparatus nor many reagents and gives a very distinct clear-cut reading.

MICROREACTION

In his most recent publication Meinicke describes the microreaction which can be carried out by the hanging drop method and requires only a platinum loop full of clear serum for the test. The fact that the specimens do not need to be inactivated has made this method practicable. The specimen can be obtained from the ear, one drop of blood being drawn up into a capillary tube of 10 cm. in length and 1 mm. in width. One end of the tube is sealed if the examination is to be made in the same place, otherwise both ends need to be sealed. After coagulation has taken place this tube is centrifuged and that part of the tube containing the clear serum is separated from the rest by means of a glass file and is then ready for use.

A cover-glass preparation is made, using five loopfuls of the diluted extract to one loopful of the patient's serum. All six drops are thoroughly mixed and the hanging drop made in the usual way. The slide is kept in the dark at room temperature for one hour, when the reading can be made by means of the microscope, using the high dry lens.

In the negative specimen the field looks almost empty, only here and there some dancing points can be seen. In the positive specimen a definite clumping with lack of motion is readily found, giving a similar picture to a positive Widal.

We have not as yet had a chance to try out this method on a large scale but consider it worthy of careful investigation.

DISCUSSION OF PAPERS BY SANFORD, CASSELMAN, KILDUFFE, OWEN, GIORDANO, KELLY, SAUNDERS

Dr. M. E. Marten.—Evidence seems to be accumulating that lipoids and cholesterin are tied up with immunity. I have found that in a large number of bloods which contain large amounts of fat, in conditions where a cholesteremia is present, e.g., syphilis, advanced nephritis, placental bloods, inhibition of hemolysis is common. Dr. H. N. Cooper's paper was very interesting and seems also to bear out some of these points. I have performed a series of experiments selecting saponin as the hemolytic agent, which I titrated against a unit volume of sheep's blood. Having obtained my hemolytic unit, I added cholesterin to a point where total inhibition or protection to lysis (for one unit volume of blood) occurred. This amount, I called the protective unit against one unit of saponin. I took a large series of Four-Plus Wassermann sera and found that far smaller amounts of protective agents (cholesterin) was necessary to prevent hemolysis. Indeed, in some reactions, practically no cholesterin was required, inhibition of hemolysis taking place even in the presence of one unit of saponin. The average amount of cholesterin required to protect against lysis in Four-Plus sera, with one unit of saponin, has not as yet been definitely determined by me; but if this should prove to be an average fixed amount, that is, a fixed or constant index, which I believe is very probable, no immune-hemolytic sera or guinea pig complement would be necessary and a means for testing positive luetic sera could be accomplished, without the use of variable reagents as ambocepter and complement.

Dr. A. J. Casselman.—The reason anticomplementary spinal fluid is not anticomplementary when mixed with serum is that normal serum has a protective action on complement. As a rule there is less fixation of complement by antigen in the presence of normal serum than there is without the serum. This fixation to which I am referring is a nonspecific fixation due to the anticomplementary properties of antigen. I have a record of some experiments which I made during the war in Paris on increasing the sensitivity of antigen. Ice-box fixation could not always be used because of occasional difficulties in obtaining sufficient ice. I found the most sensitive method to be the use of a highly anti-

complementary antigen, but I always mixed the antigen and serum and allowed it to stand for fifteen minutes, before adding the complement. Some antigens were used in such concentration that without any serum they would cause complete fixation of complement, whereas in the presence of normal serum there was no fixation of complement. Very weak positives were picked up by this method which apparently did not give false positives. Even slightly anticomplementary serums protected the complement against the anticomplementary action of the antigen to a certain extent, as these two anticomplementary factors each acted differently and there was not any marked summation of their anticomplementary properties. This method of increasing the sensitivity of the Wassermann reaction was not considered suitable for routine work in the hands of technicians, but serves to illustrate the great protective action of most normal serums on complement, when the serum is mixed with antigen and allowed to stand before adding the complement.

Dr. Henry Stewart.—It has been a great gratification to me to note the eminently fair manner in which you gentlemen have treated the Kahn test. I believe it is of value, especially in the small hospital and as a check on the Wassermann. For the obvious reason of the disproportion between the expense of time and money, and the volume of work, I do not do the Wassermann, but I paralled all that I send away with the Kahn. In several instances the question raised by a positive Kahn has resulted in the correction of a negative Wassermann report. This is not a reflection on the Wassermann or the men who did it. It is simply an exposition of the fact that all the disadvantages do not inherently lie in the small laboratory—for every individual added to the personnel increases the element of human liability to error.

For the intrinsic merit of the test, let me give you one case.

A patient with supposed carcinoma of the stomach was admitted to the house. Our diagnosis was neoplasm of the esophagus or mediastinum. He had lost one hundred pounds in less than a year. I was not able to pass the stomach tube. The dysphagia was frightful —it took him half an hour to get down his dose of barium. On the strength of a positive Kahn I began salvarsan and five days from that time he was swallowing in comparative comfort, and in a week the dysphagia had disappeared. The Wassermann report came negative; notwithstanding that, I continued the salvarsan for some weeks. There was no further improvement after the second dose, he resumed his loss of weight, which had been temporarily checked. He passed out of our hands, and died in about three months, but the dysphagia did not return.

If there were no other consideration, I feel that the euthanasia of that one man abundantly repaid me for all the time I have spent on the Kahn test.

Dr. Herman Spitz.—I have attempted the Kahn test with four specimens of cholesterin antigen sent to me by Dr. Kahn. Due to my inexperience in reading flocculation and precipitation reactions I was unable at that time to get any satisfactory results and discontinued the use of the Kahn test. One of our colleagues in Nashville is using the Meinicke test as a check on the Wassermann. He is not prepared at this time to publish the results but we have found a peculiar factor in its use with spinal fluids. Spinal fluids showing positive Wassermann were consistently negative with the Meinicke test. We accidently discovered that adding a small amount of fresh complement to the test gave a uniform positive reaction with positive fluids. This fact may have been noticed by others, I am not very familiar with the literature on this subject and am therefore not claiming anything original in this observation.

Dr. H. J. Nichols.—I was interested in Dr. Sanford's paper as I did not know before that the anticomplementary reaction had any significance, clinically. There must be several different kinds of anticomplementary sera. One of our cases, with a negative Wassermann reaction, developed an anticomplementary reaction each time he took a course of arsphenamine.

It is encouraging that there is such a close parallelism between the Kahn and Wassermann reactions. In the cases in which there is a discrepancy, it would seem as though an analysis would tell us something about that. We have had a little experience along these

lines in regard to the occurrence with reactions of the primary stage. In several instances, experimentally and clinically, the Kahn test became positive a week or so before the Wassermann did.

Dr. Robert A. Keilty.—Dr. Kolmer and I started out together in 1911. It has been my pleasure to watch his work. I think it is the consensus of opinion and I would like to say that with all the things that we have this quantitative modification of Kolmer's is the most satisfactory thing we have had in many years.

Dr. F. W. Hartman.—I want to second what Dr. Keilty has just said. There is no comeback on the clinical side of this most excellent modification every detail of which is backed up and on record. I don't think we ought to talk very much about the precipitation test until the same thing has been done in the precipitation reaction. We have not done enough on these precipitation reactions to speak authoritatively. It is only through such work that this test is going to be put on a proper basis.

Dr. Robert A. Kilduffe.—I have been very much interested in these papers. I do not think that it can be denied that syphilis is the most mishandled of all diseases and the Wassermann reaction the most misinterpreted of all laboratory examinations. To an enormous number of physicians a positive report means syphilis and a negative report means no syphilis. Those of us who work in the laboratory know that much depends upon the skill and care of the worker. The Wassermann test is a most complicated manipulation and the results may be greatly influenced by variations in technic and in technical expertness. It should be widely and consistently emphasized that this reaction constitutes only a phase of the examination for the presence or absence of syphilis and that it is the interpretation of the reaction which is of paramount importance.

Dr. John A. Kolmer.—Dr. Sanford has discussed this afternoon a subject of particular and peculiar importance and it may be well to emphasize here that Dr. Sanford has referred to the anticomplementary action of fresh serum. As is doubtless well known to all, any serum may acquire anticomplementary action after a few days; even sterile serum may undergo this change and the primary purpose for heating serum at 55 degrees C. is to remove these anticomplementary substances designated as thermolabile antilysins. However when serum is contaminated with bacteria or deeply discolored with hemoglobin, the anticomplementary action may not be materially influenced by heating and these are designated as thermostable antilysins, but Dr. Sanford has referred to thermostabile antilysins sometimes found in perfectly fresh serums. During the past two years Miss Yagle and I have noted several cases of a similar character and all were syphilitics under treatment. I regret being unable to offer anything concerning the mechanism of the reaction but I regard this as a subject of first-rate importance for investigation in serology. It is sometimes possible to test an anticomplementary serum by using a series of dilutions with and without antigen as shown in the lantern slides presented by Dr. Sanford, but when a serum is two or more weeks old it is sometimes found that the antibody has deteriorated as recently shown by Dr. Uchida in my laboratory.

It is not advisable, however, to attempt to report the degree of reaction, but to be content to report that the reaction is merely positive or negative.

I was very much interested in the paper by Dr. Casselman and particularly that portion referring to a method for reporting reactions. As far as diagnosis is concerned a report on positive, doubtful or negative would appear to be sufficient but in cases under treatment a quantitative reaction and report is usually desirable.

I thoroughly agree with Dr. Kilduffe that a primary incubation of eighteen hours in the refrigerator yields a more sensitive reaction than four hours in the refrigerator. During the past two years Miss Yagle and I have several times repeated our published experiments and the results have always indicated that the prolonged period is required for obtaining the maximum degree of sensitiveness and especially with a quantitative method. I wish a shorter period could be recommended but with a small percentage of serums the longer period has demonstrated its superiority and since the test aims to yield the maximum of sensitiveness consistent with specificity, I have no alternative but to adhere to my

original technic. I would also state that it is our practice to place the tubes in a water-bath for ten minutes after the refrigerator incubation; this is largely because of some reports indicating that occasionally syphilis reagin will fix complement better at 38° C. than at 6° C.

I am particularly pleased with the reports indicating the specificity of my test for syphilis. We have devoted considerable time to this question and regard it as specific in this country where frambesia does not occur. Even in leprosy it would appear that a positive reaction is indicative of a coexistent syphilitic infection. I think this is one of the best features of the test in order to promote confidence in the serum diagnosis of syphilis and especially in those stages of the disease when diagnosis by clinical means may not be possible. It would be an easy matter for me to render my test somewhat more sensitive, but such modifications in technic would unquestionably result in increasing the chances of producing nonspecific reactions and I believe it is better to miss the diagnosis of an occasional case of extremely latent syphilis than to fasten a false diagnosis upon a single individual.

I believe, however, that it is advisable to remove the natural antisheep hemolysin from all serums and as a result of the work of Kilduffe and of Uchida, I now routinely remove natural antisheep hemolysin because of the fact that the tests are rendered still more sensitive without danger of nonspecific reactions.

In reply to Dr. Keilty, to whom I also wish to express my deep thanks for his kind remarks and compliments, I may say that the qualitative test should always be done with 0.1 and 0.05 c.c. of serum; occasionally the 0.1 c.c. dose yields a weaker or negative reaction with syphilitic serum whereas positive reactions occur with the 0.05 c.c. amount. For this reason I have modified the qualitative test to include the 0.05 c.c. dose as originally recommended by Dr. Sanford.

I was very much interested in the paper of Dr. Owen indicating that the Kahn test may sometimes yield weakly positive reactions in the absence of syphilis. This has been my experience during the past year and especially with tests incubated overnight. In other words I believe that considerable caution should be exercised at present in the interpretation of the weakly or doubtful positive Kahn reactions with serums yielding negative complement-fixation tests, but on the other hand I regard the Kahn test as the best of the precipitation tests and as a valuable practical control test for the serum diagnosis of syphilis.

THE PHARMACOLOGY OF BENZYL ALCOHOL AND ITS ESTERS*

VI. A Clinical Investigation of the Effect of Sodium Benzyl Succinate "Benzycin" and Sodium Dibenzyl Phosphate "Benzyphos" upon the Blood Pressure in Arterial Hypertension

By Charles M. Gruber, Ph.D., M.D., St. Louis, Mo.

SINCE benzyl benzoate was introduced by Macht[1] into the realm of medicine as possessing properties of lowering the blood pressure much has been written on this subject and many new compounds have been introduced. Inasmuch as the literature has been reviewed in a previous article the reader is refered to it.[2] Among the most recently introduced benzyl compounds are sodium benzyl succinate and sodium dibenzyl phosphate. In a recent publication Gruber[3] has shown that sodium benzyl succinate produced a rise in 67 per cent and sodium dibenzyl phosphate a rise in 35 per cent of the injections

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made in experimental animals. In the remaining number either a fall or a fall followed by a rise was observed.

This work was undertaken to determine if these substances possess blood pressure lowering properties when given by mouth in clinical cases of arterial hypertension in doses designated by the manufacturers.

METHOD

The method of taking the blood pressure was the same as that employed in previous investigations[2, 4] and requires little further description here. The maximum pressures were determined with a stethoscope by the Korotkoff sounds.[5] The fourth sound was taken as the diastolic pressure. The patients were seated near a desk in a quiet room. The left arm, from which the blood pressure readings were made, rested comfortably upon the desk. Blood pressure readings were taken within two minutes after the patients had sat down and thereafter at ten-minute intervals for twenty or more minutes. These pressures were then averaged to give the results presented in the table.

The drugs were administered in tablet form. Either sodium benzyl succinate 5 grains, or sodium dibenzyl phosphate 7½ grains were given three or four times a day.

RESULTS

The results obtained upon oral administration of sodium dibenzyl phosphate and sodium benzyl succinate in clinical cases of arterial hypertension were unsatisfactory. (See table.) The observations recorded in this table were made during May and June and September and October, 1924, in the outpatient department of Washington University Medical School. The patients were kept on the same diet and the administration of fifteen drops of a saturated aqueous solution of potassium iodide three times a day after meals was continued throughout.

Upon comparing the blood pressures before and during treatment it will be seen that it remained the same or was slightly elevated by the drug. The final average of the 18 averages made from the results on the 13 patients before the treatment was systolic blood pressure 182 and diastolic pressure 100 mm. of mercury. After seven days medication this average pressure was 186 systolic and 102 diastolic. As will be seen the pressure remained practically the same and is I believe within the normal variation and error. Thirteen observations were made fourteen days after beginning medication in eleven clinical cases. The average blood pressure in these cases before medication was 179 systolic and 99 diastolic, while after having taken the drug for fourteen days they were 188 and 109 respectively.

The pulse appeared to be unchanged in most cases, in one however there appared to be a marked acceleration similar to that found in our experiments upon animals. In this case the blood pressure and pulse changed as follows:

	SYSTOLIC BLOOD PRESSURE IN MM. HG.	DIASTOLIC BLOOD PRESSURE IN MM. HG.	PULSE PER MINUTE
Before treatment	174	81	96
During treatment			
7th day	176	81	100
14th day	199	89	116
After cessation of treatment for 10 days	183	77	88

TABLE OF RESULTS

A table showing the effect of sodium benzyl succinate "Benzycin" and sodium dibenzyl phosphate "Benzyphos" upon the blood pressure in clinical cases with arterial hypertension when taken by mouth. The case numbers here correspond to the numbers used in a previous communication (4 table I). All patients gave a negative blood Wassermann except XXIV which was 4+. Only the average of the three ten-minute readings is here presented. All observations were made on the seventh and fourteenth days after the beginning of treatment except as follows: a—third day; b—fifth day; c—tenth day; d—twelfth day. Sodium benzyl succinate* was taken in 5 grain tablets and sodium dibenzyl phosphate in 7½ grain tablets three and four times a day.

CASE	BLOOD PRESSURE IN MM. MERCURY BEFORE TREATMENT	BLOOD PRESSURE SEVEN DAYS AFTER BEGINNING TREATMENT	BLOOD PRESSURE FOURTEEN DAYS AFTER BEGINNING TREATMENT
II	161– 91	*178– 98	163– 93
III	237– 98	247–103-b	232– 99-d
VIII	186– 98	174– 94-b	
	174– 94	*171– 95	
	169–100	193–103-a	188–102
IX	165–118	*169–119	157–118
XIII	215–104	205–106	193– 94
	217–105	*216–107	
	212–109	*236–119	233–118
XIX	191–109	*208–114	220–120-c
	173–106	189–114	190–116
XXI	142– 77	*157– 81	165– 85
XXIV	206–115	*200–108	
XXV	174– 81	*178– 81	199– 89
XXVI	179–107	168–107	178–105
D. A.			
F. C. 39	169–103	*174–107	
C. C.			
F. W. 38	174–106	164–111	182–101
A. J.			
M. W. 66	132– 75	*127– 73	144– 81
Average	182–100	186–102	
Average	179– 99		188–109

The temperature was found to be the same at each of these determinations. Whether the increase in pressure caused the increased pulse rate in this case or whether the increased heart rate caused an increased volume output by the heart per unit of time thus causing the rise in pressure cannot be stated.

As our previous observations upon animals[3] showed that sodium benzyl succinate caused a rise in blood pressure in 67 per cent of the injections and sodium dibenzyl phosphate in 35 per cent of the cases, that the heart is slowed in many cases, in others markedly accelerated, and that sodium dibenzyl phosphate causes a precipitate in the presence of calcium salts it would seem inadvisable to use these drugs in arterial hypertension. Intravenous administration of the latter drug would certainly not be wise.

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INTESTINAL OBSTRUCTION AND PERNICIOUS ANEMIA: REPORT OF A CASE*

By Russell L. Haden, M.D., Kansas City, Mo.

TRUE pernicious anemia is a clinical entity seemingly due to a specific toxic agent. The nature and mode of origin of the toxin are unknown. Clinical evidence strongly suggests that it arises within the gastrointestinal tract. Glossitis, diarrhea, achlorhydria, and atrophy of the gastric mucosa are quite constant findings at some stage of the disease. A diagnosis of pernicious anemia is always questionable if free hydrochloric acid is found on gastric analysis. Hartman[1] has reported two cases in which pernicious anemia followed gastrectomy. Occasionally, cases of malignant disease of the gastrointestinal tract, usually of the stomach or colon, are encountered, in which an anemia of the pernicious type occurs. It is also well known that a blood picture simulating pernicious anemia may be due to infestation of the intestinal tract by certain parasites, especially the fish tapeworm, Dibothryocephalus latus. It seems possible that in all such cases there is a toxin present, identical with that occurring in idiopathic pernicious anemia. This supposition has stimulated interest in cases of primary anemia of known etiology, in the hope that information may be gained concerning the origin and nature of the toxin responsible for idiopathic pernicious anemia.

Further evidence in favor of the gastrointestinal origin of the hemolytic toxin is found in the occasional occurrence of the clinical picture of pernicious anemia with chronic intestinal obstruction. The association of these two conditions seems more than a coincidence. The first case was reported by Knud Faber,[2] in 1895. Meulengracht[3] has recently reviewed all previously reported cases and added several which have come under his observation. Faber suggested that hemotoxic substances must be absorbed from the intestine as the result of the stasis.

On the supposition that there is some abnormal absorption as a result of stasis in the intestine, Seyderhelm[4] did an enterostomy in ten cases of pernicious anemia. He noted a rapid improvement in the anemia after operation, with a relapse after the enterostomy opening was closed. Dixon[5] has recently reported like results in a series of cases in which similar operative treatment was given. Seyderhelm[6] was able also to produce, experimentally in dogs, a macrocytic anemia, by stenosing the small intestine near the ileocecal valve.

The cases of pernicious anemia and intestinal obstruction reported by Meulengracht[3] are briefly abstracted below:

Case 1.—A woman, age seventy-five years, had had diarrhea and vomiting for several years, and a marked anemia for some time. The red corpuscles were 1.40 million and the hemoglobin 40 per cent. The red cells showed poikilocytosis and macrocytosis. Free hydro-

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chloric acid was absent on gastric analysis. The autopsy revealed an erythroblastic red marrow, and several benign strictures in the lower part of the jejunum and ileum.

CASE 2.—A woman, age twenty-seven years, complained of an intestinal disturbance for one year with irregular stools and slight vomiting. There was a progressive anemia, with a febrile period before death. The red corpuscles numbered 0.60 million and showed poikilocytosis with many macrocytes. The hemoglobin was 20 per cent. At autopsy two benign strictures 2 and 3.5 meters below the pylorus with a dilatation above the obstruction were found.

CASE 3.—A woman, aged forty-nine, had an anemia for two years. During that time there were dyspeptic symptoms, constipation and meteorism, with fever before death. The hemoglobin was 20 per cent. The red count was 0.48 million with a striking macrocytosis. There was no leucocytosis. Fourteen strictures of a fibrous nature were found in the ileum at autopsy.

CASE 4.—A male, aged twenty-eight years, complained for several years of pain in the abdomen and vomiting. The hemoglobin was 20 per cent, the red count 1.0 million and the white corpuscles 2,000. Macrocytosis was marked. The patient died following an exploratory operation. At autopsy a fibrous stricture in the jejunum, seven feet from the duodenum, was found. There was red bone marrow in the long bones. The liver, spleen, and kidneys showed an increased iron content.

CASE 5.—A woman, aged thirty-eight years, complained of swelling of the feet and legs, vomiting, and weakness. The hemoglobin was 50 per cent and the red cells 2.70 million. There was marked poikilocytosis, anisocytosis, polychromasia, and many nucleated forms. There was no free HCl in the gastric contents. The patient had slight fever, alternating constipation and diarrhea, increasing edema and anemia. At autopsy, there was a fibrous stricture in the upper part of the colon through which the finger could not be passed.

CASE 6.—A woman, age fifty-seven years, was examined in 1914, and found to have a hemoglobin of 73 per cent. She had a tendency to diarrhea, and a paresthesia of the foot. Two years later the hemoglobin was 39 per cent, and the red corpuscles 5,000. The red cells showed definite poikilocytosis, anisocytosis and macrocytosis. Gastric analysis showed no free HCl. Following this, there was some improvement in the blood condition with a subsequent relapse and death nine months later. The patient had a glossitis. The blood plasma contained bilirubin. At autopsy, there was atrophy of the papilla of the tongue, and red bone marrow in the long bones. One meter above the ileocecal valve, there was a benign fibrous stricture through which the finger could be scarcely passed.

CASE 7.—A woman, at the age of fifty-eight years, was admitted to the hospital for abdominal pain and vomiting. The gastric analysis showed the presence of free HCl. At operation a benign stricture of the intestine was found and resected. Six years later she returned, complaining of coliclike pains and a diarrhea of two years duration. A gastric analysis showed 60 acidity per cent of free HCl. The hemoglobin was 49 per cent, the red count 2.42 million and the leucocytes 4,000. Two months later the hemoglobin was 52 per cent, the red corpuscles 1.20 million, and the white corpuscles 3,400. The red cells showed anisocytosis and poikilocytosis. At operation, three benign strictures in the upper part of the ileum over a space of 0.5 meter were found. The patient died following the operation. There were abundant bacteria above the obstruction at a level at which bacteria are usually absent.

The essential facts in the case I have to report are as follows:

History.—L. H. B., a housewife, aged sixty-four, was first seen December 6, 1923, complaining of pain in the right side of the abdomen. The family history was unimportant. She had always been well except for typhoid fever and malaria many years before. The patient dated her present illness from an attack of influenza, in 1920. Following this, she began to have anorexia, eructation of gas, qualitative food distress, and constipation. She had had some attacks of pain in the gall bladder region but no colic. The attacks lasted

from one to two weeks and at times required hypodermics for relief. She stated that she had been losing weight for two weeks.

Examination.—The patient showed no evidence of marked loss of weight. There was no jaundice. In the region of the gall bladder, there was a mass extending a hands breadth below the costal margin. The mass was firm but not nodular, and quite tender on palpation. No evidence of involvement of the nervous system was found. There was some atrophy of the tongue. The examination was otherwise negative.

The fluoroscopic examination showed the stomach slightly enlarged, with some hyperperistalsis. There was no filling defect. The duodenal cap was fairly well visualized and appeared large, although no deformity was found. A radiograph of the gall bladder area was negative for calculi.

The urine examination showed only a trace of albumin. The gastric analysis revealed no free HCl and the total acidity was low. The blood examination showed 1,872,000 red corpuscles, and 4,650 white corpuscles, with a normal differential count. The hemoglobin was 51 per cent. The color index and volume index were 1.36. The red cells showed macrocytosis but very little anisocytosis or poikilocytosis. The platelets were diminished. No nucleated red cells were found.

Further Course.—The patient was given Fowler's solution and dilute HCl, and began to improve. Two weeks later she was again seen. She stated that she had been markedly constipated for one week. During that time she had also had cramplike pains in the abdomen with nausea and vomiting, and had developed a glossitis. She was admitted to the hospital December 22, 1923. For the next week she had fever, the temperature rising over 102 degrees. She was delirious at times and had involuntary bowel movements. The abdominal pain, and the vomiting continued. She was given large quantities of 2 per cent sodium chloride solution, subcutaneously. The temperature gradually fell to normal, although the other symptoms were little changed. January 15, she again began to have a slight rise in temperature, a cough with tenacious sputum and signs of consolidation of a portion of the right lower lobe. The sputum contained large numbers of Monilia. On giving potassium iodide, the signs and symptoms of pulmonary infection rapidly cleared up. The patient gradually lost weight, became progressively worse, and died February 11, 1924. During the time the general symptoms were becoming worse, the blood continued to improve, as shown in Table I. The vomiting before death was fecal in character. The chloride content of the blood was quite low. This is typical of intestinal obstruction.[7] The possible relation of the chloride metabolism of pernicious anemia has been discussed elsewhere.[8]

Autopsy.—The postmortem examination was made soon after death. Only the important findings are reported.

The body was markedly emaciated. There was a slight icteric tint to the skin. The subcutaneous fat had a peculiar lemon yellow color. The lungs were entirely negative.

There was a mass of adhesions in the gall bladder region involving the hepatic flexure of the colon, the duodenum and the pancreas. The large intestine was markedly dilated along the ascending colon and cecum with a complete constriction of the remainder of the colon. In the neighborhood of the gall bladder there was a perforation through the cecum about two inches in diameter, rather sharply defined, and opening into the large cavity. Below the perforation, the ascending colon was markedly constricted so the lumen was almost obliterated. The middle portion of the duodenum was markedly constricted and opened into the same cavity. (Fig. 1.) On passing the finger up the duodenum from the lower end, it entered the cavity. The cavity when opened, measured about six inches in diameter and contained foul smelling thick exudate. The eroded remnant of the gall bladder projected into the cavity. There were calculi in the cystic and common bile ducts. The wall of the cavity passing into the liver consisted of a zone of brown friable cellular tissue, from one to three inches in thickness, extending into the liver substance. There were isolated nodules here and there in the liver. Underneath the cavity and extending under the pancreas there were several large irregular lobulated tumor masses, suggesting enlarged lymph glands. These extended between the hilum of the liver and the pancreas. A portion of the ileum was caught in the pelvis and was distended. The mucosa showed several ir-

regular and punched out ulcers. The liver tissue, away from the tumor tissue, showed fatty degeneration especially around the periphery of the lobules.

The bone marrow from the femur was removed and showed typical grayish pink cellular bone marrow with only a few islands of fatty tissue still persisting.

Fig. 1.

The sections showed an early pneumonic reaction in the lungs. There was an increase in fibrous tissue in the portal spaces of the liver with fatty change. Sections of the tumor showed epithelial architecture with marked tendency to mucoid degeneration. Mitotic figures were very numerous.

TABLE I

BLOOD FINDINGS DURING OBSERVATION

DATE	RED CORPUSCLES IN MILLIONS	WHITE CORPUSCLES	HEMOGLOBIN	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	BLOOD CHLORIDES AS NaCl IN MG. PER 100 C.C.
Dec. 6, 1923	1.87	4650	51	1.36	1.36	1.00	550
Dec. 21, 1923	2.43	4000	53	1.09	1.22	0.90	400
Jan. 16, 1924	3.52						330
Jan. 25, 1924	3.97		80	1.00	1.01	0.99	370
Feb. 5, 1924	3.14	3800	62	1.00	0.98	1.02	405

The spleen showed a considerable increase in fibrous tissue throughout the pulp. No blood pigment was seen in the spleen. A small amount of blood pigment was seen in the central zone of the liver.

The sections of the bone marrow showed many foci of myeloid cells scattered throughout the fat tissue, presenting a picture fairly typical of a change of fatty bone marrow to red bone marrow.

The anatomic diagnosis was primary carcinoma of the gall bladder with metastasis to the regional lymph nodes and to the liver, and direct extension, with perforation, into the duodenum and the colon; malignant stricture of the hepatic flexure of the colon with dilatation of the ascending colon; partial obstruction of the duodenum, and myeloid hyperplasia of the bone marrow of the femur.

DISCUSSION

This is a case of clinically typical pernicious anemia occurring with a malignant tumor of the gall bladder and obstruction of the duodenum and colon. It is possible that the two conditions represent coincident diseases. It is most probable, however, that the toxic agent responsible for the anemia has arisen as a result of the stasis or of some alteration in function of the intestine, induced by the presence and perhaps pressure of the tumor. There is no doubt that a macrocytic anemia, typical clinically and at autopsy of pernicious anemia, may result from intestinal obstruction. This may be due to the development and absorption of hemotoxic substances within the lumen of the gut as suggested by Faber; or to some alteration in function, such as is responsible for the toxemia of intestinal obstruction. Cases of true primary macrocytic anemia are after all very rare with malignant tumors. These occur only with tumors involving the stomach and intestine. It is a question whether in such cases the toxic agent does not arise in the intestinal tract, probably as a result of the obstruction, rather than in the malignant tumor tissue.

The rapid improvement in this patient's blood condition under the treatment which benefits pernicious anemia so markedly, suggests that we are dealing here with the toxin responsible for the symptoms of idiopathic pernicious anemia. This improvement continued even with the increase in size of the primary tumor. One would not expect improvement to occur were the toxic agent arising in the tumor tissue.

The occurrence of such cases as this is further evidence of the intestinal origin of pernicious anemia.

SUMMARY AND CONCLUSIONS

Previously reported cases of pernicious anemia and chronic intestinal obstruction and the evidence suggesting a casual relation between the two conditions are reviewed.

A case of clinically typical pernicious anemia occurring with a malignant obstruction of the duodenum and colon is reported.

It is quite probable that the hemotoxic agent responsible in this case for the anemia is identical with that occurring in idiopathic pernicious anemia. The toxin possibly arises as a result of the stasis or disturbance of function within the intestine rather than from the malignant tumor tissue.

There was a marked improvement in the blood under treatment, although the tumor continued to increase in size.

The findings here are further evidence of the intestinal origin of pernicious anemia.

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ON THE USE OF RAW AND INACTIVATED SERUMS IN A FLOCCULATION TEST FOR SYPHILIS*

By C. B. McGlumphy, M.D., and W. W. Brandes, B.A., Chicago, Ill.

THE various precipitation tests for the serum diagnosis of syphilis are undergoing a thorough trial as to their practical value and doubtless the place which they are to occupy among laboratory methods will soon be definitely determined. Since the technic of the precipitation tests is such that they may be applied under circumstances that might prevent the application of the Wassermann reaction such tests may furnish valuable information to the clinician. Any modification which would further simplify or increase the sensitiveness of these reactions would be of value.

It may be mentioned that some workers would substitute certain precipitation tests for the Wassermann reaction since they believe that the precipitation methods, which are much simpler, are also more sensitive in detecting certain cases of syphilitic infection. Many who have made a comparative study of the two reactions advise the precipitation test as a check on the Wassermann test.

Epstein and Paul[1] reported a series of eleven thousand cases in which the Wassermann and Meinicke reactions were carried out simultaneously and con-

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cluded that the Meinicke reaction is more sensitive in the detection of lues in all stages, and that with the latter method false positive reactions do not occur as is the case with the Wassermann test. These workers believe that if a single laboratory test is to be used the Meinicke reaction is to be preferred to the Wassermann, but that the optimum serodiagnostic method is the application of both these reactions. A few cases in which the Wassermann and Meinicke tests gave different results came to autopsy and the gross and microscopic findings confirmed the result of the Meinicke test which was positive.

Weil and Keim[2] reported a series of cases which were tested with the Wassermann reaction and the Kahn precipitation test and came to the conclusion that the precipitation method, as checked by the clinical data, compared favorably with the Wassermann method in sensitiveness. The conclusions of these authors are cited as typical of the favorable reports on two methods which are used in many laboratories and which are based on the same general principle.

Weil and Keim heated the serums at 56° C. for one-half hour before mixing serum and antigen. Kahn[3] has recommended that the serums be heated for one-half hour at 56° C. for the purpose of removing inhibiting substances. Epstein and Paul[1] advise the use of unheated serum in the Meinicke test and state that a higher percentage of positive reactions are obtained than when the serums are heated at 56° C. for one-half hour.

In a recent paper by one of us a flocculation test for syphilis was described[4] and the results in a series of cases in which this method was used were tabulated. With this flocculation method less favorable results were obtained than with the Wassermann reaction. This was especially true of treated cases of syphilis which gave a three-plus or a two-plus Wassermann. However, a number of cases which clinically were nonluetic gave positive Wassermann and negative flocculation reactions. In this series inactivated serums were used.

For the purpose of studying the influence of heat on the flocculation reaction a small number of serums were tested before and after heating at 56° C. for one-half hour. Some of the tubes containing heated serums which gave a positive reaction showed the presence of small floccules and would be classed as weak reactions, whereas the corresponding tubes containing unheated serums showed the presence of large floccules and would be classed as strong reactions. In addition a small series of cases were studied in which the serums were heated a second time at 56° C. and the flocculation test then made in the usual manner. The interval between the two periods of heating was approximately four hours. The results in this series showed in a striking manner that two periods of heating at 56° C. weakened the reaction in a marked degree in a large proportion of the positive cases and it did not seem worth while to make further tests with serums which had been subjected to two periods of heating. Whether to use raw or inactivated serums in the flocculation test seemed to be a question of considerable importance because of the possibility of increasing the number of positive results.

The present paper deals primarily with the results obtained in a series of cases in which the flocculation test was made with serums before and after heating at 56° C. for one-half hour. That conditions would be the same for all

tests serum was removed before heating and kept under the same conditions, except for the period of inactivation, as the heated serums. A sufficient amount of the diluted alcoholic extract for both series of tests was prepared and added to both sets of serums after which they were incubated at 37° C. and readings made after eighteen hours and again after thirty-six hours. The method of preparing alcoholic extract of beef heart and performing the flocculation test has been described in a previous paper and will be outlined briefly.

PREPARATION OF ALCOHOLIC EXTRACT

Ten grams of dried and powdered beef heart extract are extracted with 90 c.c. of ether for twelve hours at room temperature. The supernatant ether is then poured off and the extraction repeated twice with fresh ether, the mixture being well shaken several times during each period of extraction. The last ether is poured off and the powder kept at 37° C. until no odor of ether can be detected after which 90 c.c. of 95 per cent alcohol are added to the container and extraction again made at 37° C. for forty-eight to seventy-two hours. The alcoholic solution is then filtered and without further preparation is ready for dilution and addition to the serums to be tested. In our experience such a procedure has always yielded a satisfactory extract.

FLOCCULATION TEST

For each test 0.1 c.c. of the alcoholic extract is thoroughly mixed with an equal amount of glycerol, 0.6 c.c. of 3 per cent sodium chloride solution added and the whole well shaken. This mixture is added to 0.2 to 0.3 c.c. of serum, the containing tubes well shaken and incubated for twenty-four to forty-eight hours at 37° C. Positive tests are recognized by the presence of white floccules of various dimensions. Negative tests show no change from the original appearance of the mixture of serum and diluted extract.

WASSERMANN METHOD

Acetone insoluble extracts of beef heart, one with and one without the addition of cholesterin, were used as antigens. Antigen, serum and complement were mixed and incubated for one-half hour at 37° C. Antisheep amboceptor and sheep cells were then added and incubation repeated for one-half hour and the results noted.

Since we were interested in studying the results of the flocculation test with raw and inactivated serums, clinical data and the results of the Wassermann reaction have been included only in those cases which showed different results before and after heating at 56° C. for one-half hour. The serums were obtained from the dispensary service of the Northwestern University Medical School in which the Wassermann test is carried out as a routine procedure. The Wassermann statistics and the clinical data were obtained from the dispensary records.

The results of the flocculation test in a series of 240 serums which were tested before and after inactivation are shown in Table I. This selected series includes all the positive cases observed during the examination of approximately two thousand serums.

TABLE I

FLOCCULATION TEST	NO.	PER CENT
Same result before and after inactivation	176	73.33
Stronger before Inactivation	37	15.41
Stronger after inactivation	7	2.91
Positive before, negative after inactivation	14	5.83
Negative before, positive after inactivation	0	
Positive before, doubtful after inactivation	6	2.5
Total number of serums	240	99.98

In analyzing the results indicated in Table I it is seen that approximately 75 per cent of the positive serums show the same result before and after inactivation. More than 15 per cent show a stronger reaction before, and approximately 3 per cent a stronger reaction after inactivation. However, the most important finding is the increased number of positive tests which occurred as a result of the use of raw serum.

In Table II are shown the results of the Wassermann test, also the flocculation test with raw and heated serums, together with the clinical diagnosis. This group comprises those cases in which there was a marked variation in the results obtained with the flocculation test before and after heating.

TABLE II

NO.	WASSERMANN	FLOCCULATION		CLINICAL DATA
		RAW SERUM	INACTIVATED SERUM	
1	++++	strong	negative	lues. myocarditis
2	–	strong	negative	lues
3	++	weak	negative	lues
4	+++	weak	negative	lues
5	++	weak	negative	no diagnosis
6	++	weak	negative	lues
7	++++	plain	negative	lues
8	++++	weak	negative	lues
9	++	plain	?	lues
10	++++	plain	negative	lues
11	++++	weak	negative	lues
12	+++	plain	?	lues
13	++	weak	negative	lues
14	–	plain	negative	tabes
15	–	weak	negative	no diagnosis
16	+++	weak	negative	lues
17	++++	weak	?	pregnancy
18	++++	weak	?	lues
19	++++	strong	?	lues
20	–	weak	?	no diagnosis

In classifying the results of the flocculation test we have followed the plan suggested in a previous paper.[4] Those tubes which show the presence of large clumps which are readily recognized with the naked eye are classed as *strong* reactions. The presence of smaller clumps which are also readily recognized macroscopically indicate a *plain* reaction, while tubes showing very small floccules which require slight magnification in order to be recognized with certainty are classed as *weak* positive reactions.

The results set forth in Table II were observed in 20 cases which gave a positive flocculation reaction before heating and a negative or doubtful reaction after heating. Of this series 4 serums gave a negative Wassermann, a

positive flocculation reaction before inactivation and a negative flocculation reaction after inactivation. One of the four cases was diagnosed clinically as lues, one as tabes, while in the third and fourth the diagnosis was not stated. Serum from the third case was reported as a two-plus Wassermann at another laboratory and in the fourth case a four-plus Wassermann was reported two years previous to the date of the flocculation test. Eleven cases which gave a positive Wassermann reaction and a positive flocculation reaction before inactivation gave a negative flocculation reaction after inactivation. Ten of the eleven cases were diagnosed as syphilis and in one the diagnosis was not stated. Five other cases which gave a positive Wassermann and a positive flocculation before heating were questionable with the flocculation reaction after heating. Four of the five cases were diagnosed as lues and one as pregnancy. In addition one case in which the diagnosis was not stated gave a negative Wassermann, a positive flocculation before heating and a negative flocculation after heating.

DISCUSSION

Of special interest is the fact that not a single serum which was negative before inactivation gave a positive reaction after inactivation. In a small percentage of the positive serums the reaction was stronger after heating, but a much greater per cent showed a stronger reaction before heating. In addition a considerable number of cases which gave a positive Wassermann reaction and were diagnosed clinically as lues were positive by the flocculation test before but negative after inactivation. Therefore, a number of cases would have been missed had only inactivated serums been tested by the flocculation method. On the other hand, no positive cases, that is serums from individuals giving a positive Wassermann and in which the diagnosis was lues, gave a positive flocculation after inactivation and a negative flocculation reaction before inactivation. As a result of these observations we are convinced that unheated serums should be used when performing the flocculation test for syphilis.

CONCLUSIONS

1. The presence of complement does not interfere with the phenomenon of flocculation which occurs when luetic serum is mixed with the ether-insoluble-alcohol-soluble components of heart muscle.

2. Many positive serums give a stronger reaction if not heated at 56° C. for one-half hour previous to carrying out the flocculation test.

3. All serums which give a positive reaction after heating will also give a positive reaction before heating.

4. A small percentage of serums which give a positive reaction before heating will give a negative reaction after heating.

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LABORATORY METHODS

A SIMPLE METHOD FOR MEASURING SURFACE TENSION CHANGES OF PURE AND BIOLOGIC FLUIDS*

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THE measurement of surface tension of biologic fluids presents certain difficulties not encountered with pure liquids, such as water, benzene, alcohol, etc. Biologic fluids are complex colloidal mixtures and frequently contain substances capable of lowering surface tension. It is the latter factor especially which may be a source of error in surface tension measurements made by means of the stalagmometer. If, for example, the surface tension of blood serum, or of plasma, is determined by the stalagmometer, the constituents which lower the tension tend to concentrate in the surface of the drop as it forms, thus permitting the formation of a greater number of drops than would be the case if the distribution of the constituents were uniform. The relatively large volume of fluid required, and the more or less troublesome technic are further disadvantages in the application of the stalagmometer to biologic fluids. The method here described meets these objections, and is believed to possess advantages over other methods.

The procedure depends upon the rise of a fluid in a capillary tube. This principle was first made use of by Frankenheim,[1] in 1847, and then by Schiff,[2] in 1884, but unfortunately, as pointed out by Sugden, was overlooked until comparatively recent times. In 1915, Lord Rayleigh[3] published a discussion of the theory of the capillary tube. Sugden,[4] in 1921, and Richards, Speyers and Carver,[5] in 1924, described apparatus making use of the same principle, but instead of employing a single tube these authors employed two capillary tubes of different diameters, and measured the difference in level of the two menisci. This has been found to be more accurate. Moreover, the simplicity and ease with which the determinations can be made are striking. The method seemed applicable to biologic fluids. I have, therefore, made a simplified form of the apparatus described by Sugden, and by Richards, Speyers and Carver, and, in addition, simplified the technic of making measurements. The results obtained with this adaptation have been satisfactory.

THE CAPILLARY U-TUBE

Two capillary tubes of different diameters are sealed together and bent into a U-tube at the point of union so that the two arms are separated about one millimeter. The diameters of the tubes I have used are 0.7348 millimeters

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and 1.920 millimeters. A hole is blown in the bottom of the U-tube to permit entrance of the fluid. The apparatus is made small enough to slip readily into a Pyrex test tube one centimeter inside diameter, and seven to eight centimeters in length. Fig. 1 shows the essential details of the apparatus.

METHOD OF MEASURING SURFACE TENSION

From 0.5 to 1 c.c. of the fluid to be measured is placed in the dry Pyrex test tube. After thorough cleansing and drying, the capillary U-tube is immersed directly into the fluid, allowing it to rest in the test tube, and the

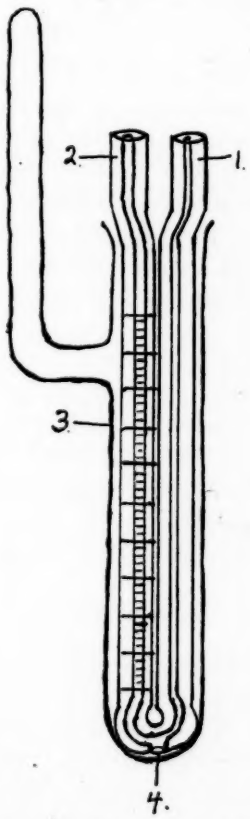

Fig. 1.—Capillary U-tube for measuring surface tension (drawn to scale).
1, Small capillary, radius about 0.4 mm.; *2*, large capillary, radius about 1.0 mm.; *3*, pyrex test tube with handle, inside diameter 1 cm., length 7 cm. to 8 cm.; *4*, opening in bottom of U-tube.

whole apparatus held vertically by a clamp on the handle in a constant temperature bath (a suitable beaker). When temperature equilibrium has been established, the fluid is drawn by suction through a small rubber tube attached to each capillary arm so as to wet the capillaries up to a height greater than the final level to which the liquid falls. The difference in level between the two menisci is read directly from the millimeter scale on the large tube, or measured by means of a pair of fine pointed calipers, which is then laid upon a millimeter scale. Readings are taken at two or three minute intervals until three successive readings give constant values. With pure

liquids, such as water, benzene, and alcohol, equilibrium, i.e., a constant level, is reached in about ten minutes. Serum and blood usually require more time.

RESULTS WITH WATER AND BENZENE

Using the apparatus described, measurements of surface tension were made on distilled water, and on a sample of Kahlbaum's benzene. The results are expressed as dynes/cm., being obtained by substitution in the following formula:

$$HGD = 2\gamma \left(\frac{1}{r_1} - \frac{1}{r_2} \right),$$

where H is the distance between menisci, G the gravitational constant, D the density of the liquid being measured, γ the number of dynes/ cm., r_1 the radius of the small capillary arm, and r_2 the radius of the large capillary arm. The radii were determined by the method employed by Richards, Speyers and Carver.[5] The values obtained on distilled water and benzene are compared with values obtained by Sugden and by Richards, Speyers, and Carver in Table I. It is seen that the results obtained with the tube here described by me agree remarkably well with those reported by these authors.

TABLE I

SURFACE TENSION OF WATER AND BENZENE

	AUTHOR	SUGDEN	RICHARDS, SPEYERS, AND CARVER
Water	72.53 dynes/cm. at 20° C.	72.59 dynes/cm. at 20° C.	75.89 at 0° C.
	" " " " "	72.81 " " " " "	70.72 " 34.32° C.
Benzene	28.49 dynes/cm. at 20° C.	28.86 dynes/cm. at 20° C.	31.87 at 0° C.
	" " " " "	28.83 " " " "	26.99 " 34.95° C.

BIOLOGIC FLUIDS

When surface tension measurements of serum are made by means of the stalagmometer it is to be expected that the observed value in dynes/cm. will be lower than the true value due to the collection in the surface of the drops of constituents lowering the surface tension. Comparison of values obtained by the stalagmometer with those determined by the capillary U-tube bear out this statement. For example, a sample of horse serum preserved with tricresol gave a value of 44.64 dynes/cm. with the capillary U-tube, and a value of 42.41 dynes/cm. with the stalagmometer. A repetition of the measurements on the same serum five months later gave 44.63 dynes/cm. with the capillary U-tube and 41.21 dynes/cm. with the stalagmometer. This discrepancy is not due to inability of the capillary method to check the stalagmometer because measurements of a sample of Kahlbaum's benzene gave 28.49 dynes/cm. with the capillary U-tube and 28.46 dynes/cm. with the stalagmometer.

Direct reading of the distance between menisci without employment of a cathetometer and reading telescope as used by Richards, Speyers, and Carver has given satisfactory results. Duplicate readings within 0.3 millimeter can easily be made. Using an apparatus having capillaries with the

radii of 0.3674 and 0.9600 millimeter and taking the values of G and D (water) as 980.4 and 0.9942, respectively, substitution in the above formula shows that a difference in level between the menisci of 0.5 millimeter corresponds to a surface tension change of 1.453 dynes/cm. Therefore, changes of at least 1.5 dynes/cm. in serum are certain to be detected with the apparatus and technic here described. The method has been successfully applied to blood, plasma, serum, and urine in this laboratory.

As illustrative of the application of the method to the detection of changes in surface tension produced by the addition of small quantities of substances to distilled water and to serum, the results in Table II are cited. The data are expressed as distance between menisci, a decrease in the distance corresponding to a lowering of surface tension and vice versa.

TABLE II

CHANGES IN SURFACE TENSION OF WATER AND BIOLOGIC FLUIDS PRODUCED BY BILE AND AGAR

Distilled water	25.0 mm.	Distilled water plus trace of bile salts	20.5 mm.
Horse serum	17.5 mm.	Horse serum plus trace of bile salts	15.0 mm.
Dog serum	17.0 mm.	Dog serum plus 0.5 mg. agar per c.c.	15.9 mm.
Oxalated pigeon blood	16.8 mm.	Oxalated pigeon blood plus trace of bile salts	16.0 mm.
Freshly voided urine	22.5 mm.	Freshly voided urine plus trace of bile salts	18.0 mm.

The advantages of the method are several and as follows: It is simple, easy, economical and accurate. Its automaticity eliminates the personal factor involved in other methods requiring drawing a wire loop through a drop or regulating the flow of a liquid. With biologic fluids errors resulting from evaporation and loss of carbon dioxide are reduced to a minimum.

SUMMARY

1. A simple apparatus and technic are described for the measurement of surface tension changes in pure and biologic fluids.

2. The method gives more reliable results with biologic fluids, such as blood serum, than are obtainable with the stalagmometer.

3. Measurement of the surface tension of distilled water and benzene gives results in good agreement with the known values for these substances.

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STUDIES IN LOCAL ANESTHESIA. IV*

The Pharmacology of Some Para-Amino-Benzoate Compounds

Local Anesthetic Action upon the Mucous Membranes and Skin of Man

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THE merit of any substance proposed for local anesthesia is determined by the standards formulated by Braun, as follows: (1) the drug must produce a diffusible, complete, and lasting anesthesia; (2) following systemic absorption, it should be less toxic than cocaine in proportion to its anesthetic power; (3) it should not produce irritation and painful infiltration or cause local tissue damage, but should be absorbed without secondary effects, such as hyperemia, inflammation, exudation or necrosis; (4) it should be soluble in water and its solutions should be stable; (5) it should be readily sterilizable by heat, preferably by boiling in solution; (6) unless more powerfully anesthetic and at the same time less toxic than any known substances, the drug should be compatible in solution with adrenalin. For several years novocaine has most nearly fulfilled these requirements.

In the experimental determination of local anesthetic power, several methods have been proposed. The comparative value of these varies in the hands of different investigators and widely divergent results have been reported. In general, experiments performed upon laboratory animals show greater variation and are of less value than those performed upon man. Animal experimentation is of great value in the determination of toxicity and local effects upon tissues, but the ultimate efficiency of any new drug will then depend upon its action in practical use.

The purpose of the present investigation is to determine their anesthetic potential by experimentation upon the mucous membranes and skin of man.

A. SURFACE ANESTHESIA OF THE TONGUE BY IMMERSION

Anesthesia of the buccal mucous membranes was one of the earliest local anesthetic effects to be observed. The sensation of numbness resulting from chewing coca leaves suggested the use of cocaine as a surface anesthesia. The surface anesthetic power of new drugs may be demonstrated by applying them to the tongue or gums. The anesthetic action is manifested by a sensation of numbness very easily recognized. The experiment is essentially the same as clinical practice.

Method.—I have described the method elsewhere.† The tip of the tongue was immersed in solutions of the drug in physiologic saline for five minute peri-

*From the Departments of Surgical Research and Pharmacology, University of Illinois. The author wishes to express his thanks to H. A. McGuigan, W. J. R. Heinekamp, and S. J. Cohen for valuable suggestions and for affording him the use of their laboratory facilities.

†Jour. Lab. and Clin. Med. (In Press.)

ods. When effective the tip of the tongue usually felt numb at the end of two minutes. The mouth was then rinsed out at the end of five minutes and the tip of the tongue tested for anesthesia by rubbing it against the cutting margins of the incisor teeth. Cocaine and butyn were used as controls. All tests were made upon my tongue. Sufficient time was allowed between tests for complete recovery of sensation when the solutions were anesthetic or when a distorted sensation resulted.

Results.—The comparative activity of these substances in both 2 and 1 per cent solutions is shown in the first two columns of Table I. The superiority of butyn to cocaine is at once manifest, the saturated solution of butyn (slightly less than 1 per cent) being as powerful as 2 per cent cocaine. Butyn is thus more powerful in surface anesthesia than any of the new drugs. A2 and B1 have no surface anesthetic power. B4 is anesthetic in saturated solution, which is between 0.25 and 0.5 per cent at room temperature. It is unfortunate that this drug is not soluble in higher concentrations as it is powerfully anesthetic. A5, B2, and B3 are anesthetic for a brief period in 2 per cent strength but ineffective in 1 per cent solution. A3, A4, A7, and B5 are easily the equals of

TABLE I

COMPARATIVE ACTIVITY OF DRUGS UPON THE TIP OF THE TONGUE. THE NUMBERS REPRESENT THE DURATION OF ANESTHESIA IN MINUTES

	THE DRUGS ALONE		2% BICARBONATE 2% OF DRUG
	2% STRENGTH	1% STRENGTH	
Cocaine	11	5-6.	
Butyn*		10.	
A2	0	0	
A3	11	6	11
A4	14	4	7
A5	5	?	15
A6	Irritating		
A7	12	4	8
B1	?	0	
B2	7	0	22
B3	5	0	18
B4†		5	
B5	11	5	22
B6	Irritating		

*Saturated solution somewhat less than 1 per cent.
†Saturated solution between 0.25 and 0.5 per cent.

cocaine. A6 and B6 were irritating in other experimental work. They exerted a biting, stinging effect upon the tongue lasting a few minutes, which further confirmed their irritant qualities.

The effect of the addition of sodium bicarbonate was next studied. In surface anesthesia it has been shown that the bases of local anesthetics are more effective than their salts. The addition of an equal amount of alkali to a novocaine solution increases the surface anesthetic power almost to that of cocaine. This is explained by the liberation of the free bases which penetrate the mucous membranes more readily than the water soluble salts.

Sodium bicarbonate was added to A3, A4, A5, A7, and B2, B3, and B5 in the same concentration as the anesthetic drug. With the exception of A5 and B2 all solutions became cloudy. A3, A4, and A7 became milky in appearance,

straw-colored, oily globules later floating on top. Focculi then appeared and later a curd-like precipitate. A4 and A7 were much less anesthetic at this stage and A3 just the same, thus showing incompatibility in solution with alkali.

The anesthetic power of B2 was markedly increased. B3 became opalescent but remained powerfully anesthetic. B5 was opalescent during the first six hours with a marked increase in anesthetic power. Twenty-four hours later oily globules separated out. While A4, A3, and A7 were incompatible with alkali after six hours, it is likely greater anesthetic power would have been observed just after the addition of the bicarbonate. The increase in anesthetic power by this means, when compatible, is roughly three times in terms of duration.

All tests for surface anesthesia of the skin were negative. The 2 per cent mixtures of the drugs with bicarbonate were applied to the skin for five minutes by means of a cotton applicator. The tests were repeated, dipping the applicator in crystals forming a paste. Finally the applicator was dipped in a mixture of equal parts of crystals of the drug and sodium bicarbonate. No anesthetic effect was produced by any of these methods.

B. ANESTHESIA OF HUMAN SKIN BY DERMAL WHEALS

Anesthesia of the skin by dermal wheals is an attractive method because it is identical with clinical usage. This method involves direct action on the terminal nerve filaments and sensory end-organs of the skin. Anesthesia is but very little dependent on pressure within the layers of the skin because wheals of physiologic salt solution do not produce anesthesia. Anesthesia, therefore, results from a direct chemical action upon the nerve endings. The great advantage of this method over others was first emphasized by Braun. He determined anesthetic potential both by duration and by minimal anesthetic concentration.

Method.—The thighs and anterior abdominal wall were closely shaved. Dermal wheals were then raised with the Meeker syringe and finest hypodermic needles freshly sharpened. The needle was thrust beneath the skin surface with the bevel downward. At the moment the needle point entered the epidermis, injection began, which was always endermic and not subcutaneous. Dermal wheals were thus made to stand up from the surrounding skin like urticarial wheals. Wheals were one-half of an inch in diameter and required one-fourth of a cubic centimeter of solution each. It is important that all wheals be as nearly the same size as possible and contain the same amount of solution, all of which has been injected intracutaneously. Adequate controls were employed, consisting of novocaine, cocaine, butyn, and salt solution, so that disturbance of sensation was not interpreted as anesthesia. Progressive series of dilutions in physiologic salt solution were injected, as one-fourth, one-eighth, one-sixteenth, one-thirty-second, one-sixty-fourth and one-one hundred and twenty-eighth of 1 per cent.

I made all wheals upon myself. The skin of the thighs and abdominal wall is of such thickness that accurate wheals may be raised painlessly when the substance is anesthetic. The sensitiveness of the skin and the rapidity of absorption vary in different areas of the body. It also varies in different individ-

uals, depending upon familial traits, exposure, vocation, etc. By employing the same skin areas in the same individual these factors remained constant. The duration of anesthesia in the same cutaneous area may also be shortened by previous brisk massage, heating, or muscular exercise because of the improved circulation and consequently more rapid absorption. In these tests the patient remained seated and sources of external heat were avoided.. Wheals were marked with a circle of mercurochrome as soon as raised so that the center of the endermic infiltration was easily identified for testing after the wheal

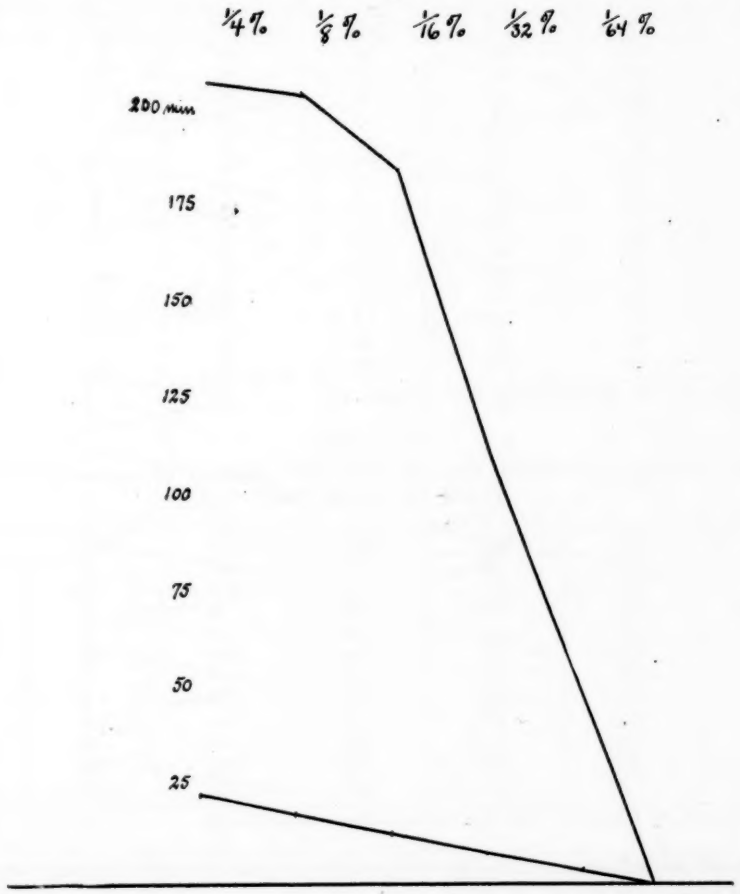


Fig. 1.—Diagrammatic representation of anesthetic power. Duration expressed by the base line of the isosceles triangle and minimal anesthetic concentration by the apex.

had disappeared. Tests for sensation were made by scratching the area with a wooden applicator or with a needle as in vaccination.

Results.—Table II expresses both duration of anesthesia and minimal anesthetic concentration. The minimal anesthetic concentration of cocaine and butyn was one-thirty-second of 1 per cent. Both were ineffective in one-sixty-fourth per cent strength. The minimal anesthetic concentration of novocaine is one-sixteenth of 1 per cent. A2 is the equal of novocaine. A5, A6, and B1 are only slightly better. A3, B2, and B3 are the equals of cocaine. A4 is

slightly more powerful and A7, B4, B5, and B6 distinctly more powerful than cocaine and butyn. These relationships are expressed graphically in Fig. 1.

The stability of these drugs in solution was not thoroughly tested. They had been in solution a week before these tests were begun. After determination of anesthetic power in unsterilized solutions they were all boiled briskly for five minutes and the same tests repeated. No difference in anesthetic power

TABLE II

COMPARATIVE ANESTHETIC ACTION IN DERMAL WHEALS. NUMBERS REPRESENT DURATION OF ANESTHESIA IN MINUTES

	1/4%	1/8%	1/16%	1/32%	1/64%	1/128%
Cocaine	21	16	10	6	0	
Butyn	22	15	11	7	0	
Novocaine	16	10	5	0		
A2	15	12	7	0		
A3	21	16	10	4	0	
A4	23	18	11	5	3?	0
A5	19	12	7	4	0	
A6*	16	10	7	4	0	
A7	23	20	18	11	5	0
B1	18	13	7	0		
B2	21	15	14	8	0	
B3	21	15	11	6	0	
B4	24	21	17	12	6	0
B5	25	21	14	10	4	0
B6*	25	21	15	10	4	0

*A6 and B6 are too irritating for clinical use.

TABLE III

COMPARATIVE ACTIVITY IN DERMAL WHEALS WITH THE ADDITION OF ADRENALIN, 10 MINIMS TO 100 C.C. OF SOLUTION*

	1/4%	1/8%	1/16%	1/32%	1/64%	1/128%
Cocaine	205	195	170	100	0	
Butyn	180	160	73	100	15?	0
Novocaine	155	130	50	0		
A3	160	140	95	50	0	
A4	180	170	150	90	60	0
A5	200	190	85	30	0	
A7	105	105	55	25	0	
B2	250	250	190	90	34	0
B3	200	210	200	80	20	0
B4	200	200	200	170	90	10?
B5	210	200	200	130	85	0

*The numbers represent duration of anesthesia in minutes.

was observed which was not easily within the limits of experimental error. The drugs will thus stand a limited amount of boiling in solution without deterioration.

Effect of Adrenalin.—Adrenalin by itself is not anesthetic, but added to solutions of novocaine and cocaine it increases the duration of the anesthesia from four to ten times. Tropacocaine is the only drug uninfluenced by the addition of adrenalin. Its action is not true synergism because the minimal anesthetic concentration is not altered. The effect has been repeatedly shown to be due to the vasoconstriction of the adrenalin, which has earned it the name of the chemical tourniquet.

Adrenalin solution (1:1000) was added to the anesthetic solutions in the proportion of 10 drops to one hundred cubic centimeters (three drops to the

ounce) just before using. When the control solutions had turned the slightest shade of pink they were discarded and fresh ones made.

The addition of adrenalin produced intense blanching in the wheals which often outlasted the effects of the anesthetic. The adrenalin in all instances greatly prolonged the anesthesia, as is seen in Table III. A2 and B1 were not included because of their lower anesthetic power. A6 and B6 were not included because of their irritating qualities. There is a greater variation in the end-point by this method so that it is not appropriate for quantitative comparisons of anesthetic power. Variations of from half an hour to an

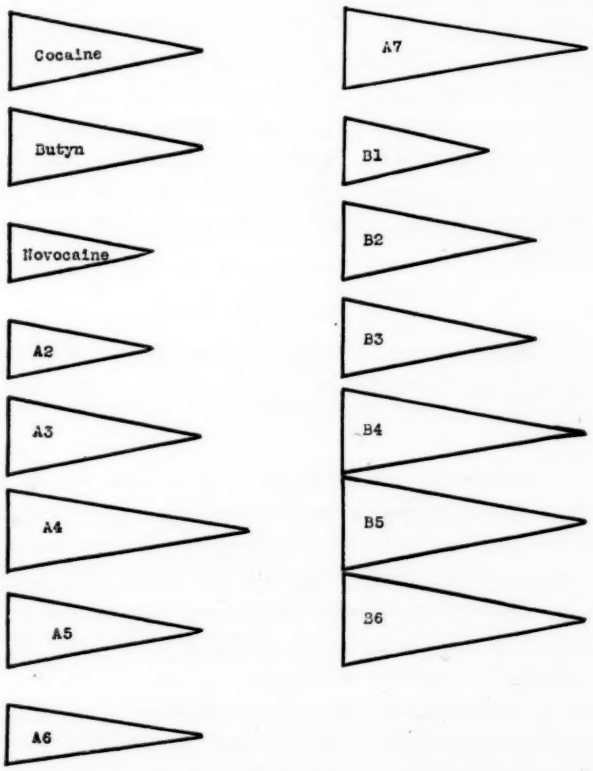


Fig. 2.—Curves representing anesthetic activity with and without adrenalin. Duration of anesthesia is shown on the vertical line and dilutions on the horizontal one. By the addition of adrenalin, anesthesia is prolonged 8 times and the more effective concentrations, ¼, ⅛, and 1/16 per cent tend to become equalized in duration.

hour are not uncommonly seen in repetitions due to unexplainable factors. These results would therefore show considerable variation if repeated.

The duration of anesthesia when adrenalin is used depends much less upon percentage strength of the drug, providing it is an effective concentration. The fourth, eighth, and sixteenth per cent strengths are very nearly equal in duration of anesthesia, for the same amount of adrenalin was used in each. Much weaker solutions should be employed in practical use when adrenalin is added. This relationship is expressed by the graphs in Fig. 2, which represents the average anesthetic action of A4, B2, B3, and B5, with and without adrenalin.

SUMMARY

The results of experiments thus far indicate that all these drugs are stable in solution, that they do not deteriorate by boiling for a limited time, and that they are compatible in solution with adrenalin.

A1 should be discarded because it has not sufficient anesthetic power. A2 and B1 may be discarded for the same reason, since they are no better in this regard than novocaine. (No. 2 is novocaine.)

A6 and B6 are unfit for practical local anesthesia because they are marked local tissue irritants.

For surface anesthesia of mucous membranes A3, A4, A7, and B5 are at least as powerful as cocaine, and give promise of usefulness in this field. B4 is not sufficiently soluble. The others have not sufficient penetrating power.

For infiltration anesthesia A3, A5, B2, and B3 are as powerful as cocaine. A4, A7, B4, and B5 are more powerful than cocaine, and promise to gain favor over the present drugs employed in this work. Their future usefulness in infiltration anesthesia will depend upon systemic toxicity and local tissue reaction. Further work along these lines is necessary before their fitness for clinical use is determined.

Local Tissue Reactions

By William R. Meeker, M.D., Chicago

AMONG the requirements which Braun[1] prescribes for a local anesthetic drug, a very important one is that it should not produce local damage to tissues. One of the common objections offered by the opponents of local anesthesia is the occasional interference with healing which they ascribe to the local action of the drug. Several proposed substitutes for novocaine have been more powerfully anesthetic but have failed to gain favor because of the local tissue irritation and damage which they produce.

The absence of irritating qualities in novocaine is marked even when applied in concentrated solution or powder form to sensitive wounds or on such delicate surfaces as the cornea. Injected within the tissues it exerts but little influence on the vasomotor nerves of the part. The injections are without pain and free from irritation. There is no after-pain, inflammation, persistent hyperemia, or induration.[2]

Cocaine may be ranked with novocaine in its action upon tissues. It produces vasoconstriction when applied locally and after injection into tissues, which results in blanching without preliminary burning or dilatation of vessels. Injections are painless and there are no harmful postanesthetic effects. When properly employed, therefore, the healing process is delayed only until the solutions are absorbed, after which it proceeds normally.

Quinine urea hydrochloride is an example of a local anesthetic which produces marked local tissue disturbances. Solutions of sufficient strength produce an anesthesia lasting for hours or even days, the prolonged anesthesia depending upon tissue damage. Hertzler[3] states that an exudate is produced

which is at first amorphous and later coagulates forming granular fibrin. A wheal thus produced becomes hyperemic and indurated, the fibrin being absorbed within one or two weeks with but slight replacement by fibrous tissue. Often in clinical work the parts assume a dark ecchymotic appearance which sometimes proceeds to coagulation necrosis and sloughing. Such a potential interference with healing is a serious drawback.

The local irritant action is also manifested by the production of pain during the injection. This is so marked that in rectal work Allen recommends the preliminary injection of Schleich's solution to prevent the burning pain during and immediately after the injection of quinine salts. An anesthetic which first elicits pain after injection has been called "anestheticum dolorosum." This is a manifestation of an irritant chemical reaction. For these reasons quinine urea hydrochloride has been largely abandoned for infiltration anesthesia and it never was popular in nerve block methods.

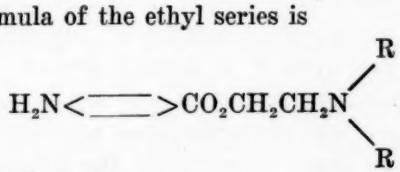
Alcohol is an example of a more marked tissue irritant or anestheticum dolorosum. Infiltration with it is exceedingly painful and the resulting anesthetic effect due more to destructive tissue change than to a specific anesthetic action upon nervous elements. Wheals of 50 per cent alcohol are followed by gradual sloughing of the center after a week or ten days.

NEW ANESTHETIC DRUGS

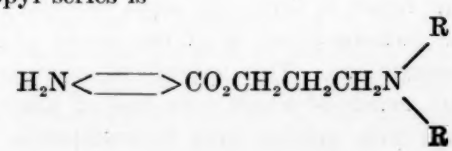
The drugs comprising the subject of this investigation were prepared and furnished by Dr. Roger Adams of the Department of Chemistry of the University of Illinois. They are divided into two groups, an ethyl and a propyl series, as follows:

	ETHYL SERIES	MOL. WT.
A-I	Dimethylamino ethyl para-amino benzoate hydrochloride	244.5
A-II	Diethylamino ethyl para-amino benzoate hydrochloride	272.5
A-III	Di-n-propylamino ethyl para-amino benzoate hydrochloride	300.5
A-IV	Di-n-butylamino ethyl para-amino benzoate hydrochloride	328.5
A-V	Di-isopropylamino ethyl para-amino benzoate hydrochloride	300.5
A-VI	Di-isobutylamino ethyl para-amino benzoate hydrochloride	328.5
A-VII	Di-secondary butylamino ethyl para-amino benzoate hydrochloride	328.5
	PROPYL SERIES	
B-I	Diethylamino propyl para-amino benzoate hydrochloride	286.6
B-II	Di-isopropylamino propyl para-amino benzoate hydrochloride	314.6
B-III	Di-normal propylamino propyl para-amino benzoate hydrochloride	314.6
B-IV	Di-normal butylamino propyl para-amino benzoate hydrochloride	342.7
B-V	Di-iso-secondary butylamino propyl para-amino benzoate hydrochloride	342.7
B-VI	Di-isobutylamino propyl para-amino benzoate hydrochloride	342.7

The general formula of the ethyl series is



That of the propyl series is



Other investigations have shown some of these preparations to be wanting in certain essentials which eliminates them as desirable anesthetics. A-I was found to be too weakly anesthetic for further consideration. A-VI and B-VI were found to be markedly irritating to rabbits' eyes even to the point of corrosion. Of the remainder A-II and B-I are least anesthetic, being no more powerful in this respect than novocaine. The present investigation, therefore, includes more particularly numbers A-III, A-IV, A-V, and A-VII and B-II, B-III, B-IV, and B-V.

The methods employed were the determination of: (1) Gross changes in dermal wheals on dogs, using the more concentrated solutions; (2) Gross changes following injections of the more concentrated solutions into rabbits' ears; (3) Gross changes and sensations produced by dermal wheals and irrigation of the conjunctival sac in man; (4) Microscopic changes following intradermal infiltration in the dog. Wherever possible controls of novocaine and cocaine were used because of their lack of irritant qualities, and quinine urea hydrochloride and alcohol were included because of their marked local irritation.

I. *Changes in the Skin of Dogs.*—Two series of dermal wheals were raised on the backs of two dogs. The solutions were of 2 per cent strength with the exception of B-IV which was a saturated solution. Within half an hour there was a yellowish discoloration of A-IV, A-VII, and the quinine urea hydrochloride. The center of the alcohol wheal was white surrounded by a hyperemic zone. One day later these wheals were marked by brick red discolorations resembling crusts, although they were really intracutaneous. Within ten days to two weeks these areas became crusts and were later cast off representing a superficial necrosis. Hyperemic areas persisted after the desquamation of these crusts. These changes were more marked with alcohol, decreasing in severity with A-VII, A-IV, and quinine urea hydrochloride, in the order named. There was no manifestation of local injury with the other drugs in this test.

II. *Injections into rabbits' ears* with 2 per cent solutions proved valuable in determining the amount of induration as well as other gross changes. One-half of a cubic centimeter of 2 per cent strength was injected in each case. After an interval of a day A-IV and A-VII were characterized by marked thickening. The same feature was observed to a less extent with quinine urea hydrochloride, butyn, and B-V. These areas were gradually resorbed within ten days without necrosis.

III. *Dermal Wheals and Conjunctival Irrigations in Man.*—The use of dermal wheals on man is a convenient method of studying local irritation as well as anesthetic power, providing it is already known from animal experimentation that the concentrations employed will not be escharotic. I placed two series of wheals of 2 per cent strengths upon my thigh. A-IV and A-VII became discolored as in the dog. In addition the stratum corneum became raised after twenty-four hours to form fair sized blisters. Later the areas became brick red in color forming crusts after two weeks which persisted beyond the limits of these observations. This superficial necrosis was more marked with 50 per cent alcohol, crusts of which were case off after three weeks. Such changes are less marked with quinine urea hydrochloride.

Painful infiltration was marked only with alcohol, quinine urea hydrochloride and to a much smaller degree with A-IV. In order to accentuate the pain-producing qualities, solutions were made in distilled water. Infiltration with these solutions was painless even in hypotonic solution, as one-fourth per cent strength. All of the new drugs are therefore true anesthetics and cannot be classed with quinine and alcohol as anesthetica dolorosa.[4]

One-half per cent strengths were used in irrigations of the conjunctival sac. A-IV and A-VII were markedly irritant. Irrigation was followed at once by a burning, smarting, and aching pain until the solutions were washed out. Marked injection of the conjunctiva persisted. There was mild transient smarting with quinine urea hydrochloride, which was less marked with butyn and B-V. Very slight hyperemia followed B-V. The others were entirely negative.

IV. *Microscopic Changes.*—Dermal wheals of large size were raised with 2 per cent strengths in salt solution usually injecting one-half of a cubic centimeter to each wheal. After twenty-four hours the dog was killed and the wheals excised. Microscopic sections were made according to the usual pathologic technic and stained with hematoxylin and eosin.

All wheals were somewhat indurated to palpation, A-IV and A-VII being more marked. In man the induration of these two wheals and those of quinine and butyn lasted longer than that of the other drugs.

The epithelial layers of the epidermis were apparently normal. While in man it is necessary to infiltrate between the epithelial layers to produce the charactristic raised, orange peel appearance of a wheal, the epidermis is much thinner in the dog so that infiltration between the epidermis and corium produces typical raised wheals. The infiltrated area proper included only corium, extending from the epidermis as deep as the bases of the hair follicles. The infiltrated area was surrounded by a zone of reaction comprising contiguous subcutaneous tissue. Three separate layers or zones were thus recognizable in all wheals. From the surface these layers were: (1) epidermis, (2) infiltrated area of the corium, and (3) the reaction zone of surrounding subcutaneous tissue.

The infiltrated area was easily recognized in all wheals. Tissue spaces seemed larger than normal, fibers being spread apart and arranged in loose meshwork. Individual strands were much enlarged, probably from imbibition of infiltrated fluid, and the entire area stained a dark pink color. There was a general hyaline appearance in which the finer structural details were lost.

Tissue changes varied from complete superficial necrosis to only a slight round cell infiltration. Cocaine and novocaine showed no coagulated exudates in the infiltrated zone and only a mild lymphocytic infiltration in the subcutaneous zone of reaction. In both instances a few lymphocytes had found their way into the infiltrated area. These are examples of mild tissue reactions.

Butyn, quinine urea, and alcohol produced more severe local tissue changes. In the butyn wheal the connective tissue strands were more thickened. There was a very marked infiltration of lymphocytes in the surrounding subcutaneous tissue, which also permeated the area of anesthetic drug infiltration. These changes were equally as marked in the quinine urea wheal and there were also small granular areas of coagulated exudate in the reaction zone of the subcu-

taneous tissue. There was marked hyalinization of the infiltrated zone of the wheal of 50 per cent alcohol. A fine granular material representing coagulated exudate was also present between strands of fibrous tissue. Cellular infiltration was not marked.

Local tissue disturbance may thus be estimated upon (1) the swelling and hyalinization of connective tissue strands of the infiltrated corium; (2) the presence of transudates and exudates; (3) the infiltration with lymphocytes and leucocytes. The extent of irritation of the new drugs may be estimated as follows:

A3 +++.	B2 +++.
A4 +.	B3 +.
A5 ++.	B4 ++.
A7 +++.	B5 +++.

SUMMARY

The criteria of tissue disturbances employed show considerable variation in irritant action. All the new drugs tested were true local anesthetics in that anesthesia was never preceded by pain during infiltration. Gross tissue changes of dermal wheals show A-IV and A-VII to be sufficiently corrosive to produce superficial necrosis. This action was also manifest on rabbits' ears; B-V also appeared irritating. In the conjunctival sac one-half per cent solutions of A-IV and A-VII were markedly irritating and B-V mildly irritating. The other drugs caused no disturbances. Microscopic study of excised wheals further confirms the irritant action of A-VII and B-V and show A-III and B-II to be equally disturbing.

A consideration of all tests warrants the discarding of A-IV, A-VII, and B-V. It seems certain that these drugs would cause interference with healing in clinical practice. Which of the remaining drugs deserve a clinical trial will depend upon systemic toxicity. This is of extreme importance in many of the regional anesthetic procedures where the injection of large amounts of the anesthetic drug is necessary. The low toxicity of novocaine has been largely responsible for the development of regional anesthesia to its present high status. Other features being equal, the drug replacing novocaine in regional anesthesia must either be more powerfully anesthetic in proportion to toxicity, or less toxic in proportion to anesthetic power, thus affording a wider margin of safety when large amounts must be injected.

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STUDIES IN LOCAL ANESTHESIA. V

The Toxicity of Para-Amino-Benzoate Compounds*

By H. McGuigan, Ph.D., M.D., and Glenn A. Brough, M.S., M.D.,
Chicago, Ill.

THE following experiments were carried out to determine the toxicity of the compounds of this series by injection into the peritoneal cavity of albino rats. This was chosen because rats were available, and also because the conditions approximate clinical application more nearly than if cold-blooded animals had been used. The method used was as follows:

The rats were weighed. The dose was given in milligrams per kilogram body weight. Since the solutions were equimolar, the same volume of each solution in cubic centimeters was given to rats of the same weight. The molecular concentration corresponded to 1 per cent procaine. The solutions were kept at body temperature by an electric thermostat, and all intervals were timed with the stop watch.

The animal was held; injection was made into the peritoneal cavity, and the animal immediately turned loose. The symptoms of toxicity were practically the same in all cases, regardless of the drug used. The rat soon became restless, excitable, and decidedly irritable. A touch on the tail at times caused a very definite jump. There were four stages in the symptoms as follows: first, increased irritability; second, paralysis; third, convulsions; fourth, coma and death. The onset of each of these was more or less abrupt, and in the case of the convulsions and death was easily determined.

The rats after the short irritable excitable stage, began to show signs of paralysis. The wheezy dyspneic character of the respiration, accompanied by gnashing of the teeth, became more and more marked, while the hind legs became slowly paralyzed. Although the legs were motionless, a painful stimulus caused the rat to squeal and attempt to drag them away. The other reflexes throughout were positive. The clicking or gnashing of the teeth and the dyspnea continued, the neck became arched backward, and the animal suddenly went into a convulsion which lasted a few seconds, and was followed by another after a short interval. During the spasm the animal was in opisthotonos, but in the interval it relaxed with the exception of the neck muscles. This point of relaxation was not true in the later stages, where exhaustion seemed to supervene. In some instances when large fatal doses were given, the spasms were tonic instead of clonic in type. They usually ended in death without remission. The accessory respiratory muscles were active after the breathing became difficult.

Death occurred from a respiratory failure, the heart continuing to beat for about one minute after cessation of respiration. Thus two definite points

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were taken as a measure of the relative toxicities—the time of the onset of the convulsions, and the time of death.

Animals which survived the convulsions for periods of twenty to thirty minutes usually recovered, regardless of how severe the convulsions had been, or the length of the coma folowing the convulsions, which might have been an hour or more. The following morning the rats were apparently normal. In some cases weakness of the hind legs was marked and persisted for twenty-four hours or longer.

Table I gives (A) the dose that will cause convulsions, (B) the fatal dose, (C) the numbers of the drug which cause these symptoms with any given dose.

TABLE I

DRUG A	MINIMUM MG. PER KILO CAUSING CONVULSIONS	AV. TIME IN MIN. OF ONSET OF CONVULSIONS.	NUMBER OF ANIMALS USED	NUMBER HAVING CONVULSIONS	MINIMUM MG. PER KILO CAUSING DEATH	AVERAGE TIME IN MINUTES—SECONDS	NUMBER USED	NUMBER OF DEATHS
I	350	7'45"	3	1	non toxic		3	0
II	150	5'25"	17	7	150	6'45"	17	5
III	75	3'50"	4	3	100	4'15"	10	5
IV	25	8'00"	3	1	50	7'20"	5	2
V	75	4'05"	2	1	100	3'10"	5	1
VI	75	2'50"	2	1	100	5'10"	4	2
VII	75	2'25"	2	1	100	3'00"	4	2

TABLE II

SERIES B. (BY S. J. COHEN)

	DOSE PER KILO THAT KILLS BY CARDIAC AND RESPIRATORY PARALYSIS	TIME TO KILL HR.	TIME TO KILL MIN.	DOSE PER KILO FROM WHICH THEY RECOVER
B 1	125 mg.	5	00	100 mg.
B 2	75 mg.	4	15	50 mg.
B 3	100 mg.	7	25	75 mg.
B 4	75 mg.	2	55	50 mg.
B 5	100 mg.	2	50	75 mg.
B 6	200 mg.	15	00	150 mg.
order				
B 4	75 mg.	2	55	
B 2	75 mg.	4	15	
B 5	100 mg.	2	50	
B 3	100 mg.	7	25	
B 1	125 mg.	5	00	
B 6	200 mg.	15	00	

TABLE III

MINIMAL LETHAL DOSE OF COCAINE IN WHITE RATS. INTRAPERITONEALLY (COHEN AND MCGUIGAN)

NUMBER OF RATS USED	AMOUNT OF DRUG	TIME OF CARDIAC STOPPAGE HR.	TIME OF CARDIAC STOPPAGE MIN.	REMARKS
2	100 mg. per k.	4	50	Both died
1	75 mg.	3	05	Died
6	50 mg.	6	06	3 died 3 recovered
2	25 mg.	Recovered		Both recovered

TABLE IV

	SERIES A					COCAINE EQUIVALENTS (COCAINE = 1)		
	DURATION OF ANESTHESIA IN RABBIT'S EYE	QUADDEL METHOD. AMT. OF DRUG IN 1 C.C. TO PRODUCE ANESTHESIA IN 5 MIN.	TIME NECESSARY TO PRODUCE PARALYSIS OF THE SCIATIC NERVE IN FROG	PHENOLIC COEFFICIENT. TIME NECESSARY TO KILL BACTERIA IN 1% SOLUTION	TOXICITY. MINIMUM AMT. PER KILO BODY WEIGHT OF DRUG PRODUCING DEATH IN WHITE RATS	COCAINE VALUES IN EYE	ON SCIATIC NERVE PARALYSIS	ON FROG'S SKIN (TÜRCK METHOD)
I	none	–	13′ 57″	–	nontoxic	none	0.25	0
II	14 min.	10 mg.	5′ 30″	9 hr.	150 mg.	0.20	0.16	0
III	16 min.	7 mg.	1′ 42″	5 hr.	100 mg.	0.25	1.7	1
IV	30 min.	19.5 mg.	1′ 30″	2.5 hr.	50 mg.	0.62	2.0	0.8
V	23 min.	15 mg.	1′ 5″	4.5 hr.	100 mg.	0.44	3.0	0.4
VI	45 min. very irritant	12.5 mg.	3′ 55″	(0.15%) 5 min.	100 mg.	2. +	0.25	0.3
VII	46 min.	7.0 mg.	2′ 0″	45 min.	100 mg.	2. +	2.0	0.6
Cocaine		6.0 mg.			50 mg.			

TABLE V

	SERIES B					COCAINE EQUIVALENTS		
	DURATION OF ANESTHESIA IN RABBIT'S EYE	QUADDEL METHOD. AMT. OF DRUG IN 1 C.C. TO PRODUCE ANESTHESIA IN 5 MIN.	TIME NECESSARY TO PRODUCE PARALYSIS OF THE SCIATIC NERVE IN FROG	PHENOLIC COEFFICIENT. TIME NECESSARY TO KILL BACTERIA IN 1% SOLUTION	TOXICITY. MINIMUM AMT. PER KILO BODY WEIGHT OF DRUG PRODUCING DEATH IN WHITE RATS	COCAINE VALUES IN EYE	ON SCIATIC NERVE PARALYSIS	ON FROG'S SKIN (TÜRCK METHOD)
I	26 min.	20 mg.	2′ 32″	2.5 hr.	125 mg.	0.50	0.5	0
III	35 min.	18.5 mg.	2′ 8″	3 hr.	100 mg.	4.	0.4	0.7
IV	42 min.	2.5 mg.	1′ 15″	(⅓%) 5 min.	75 mg.	0.75	0.9	0.2
II	60 min.	15 mg.	3′ 0″	3 hr.	75 mg.	1%	2.7	1.0
V	63 min.	17 mg.	1′	(0.5%) 5 min.	100 mg.	4	1.3	0.9
VI	Too irritant	21 mg.	2′ 20″	(0.2%) 5 min.	200 mg.	Too irritant for use	0.7	0.9

There is some slight variation in the tables which is very obviously due to differences in the rate of absorption, e.g., the doses causing convulsions under (C). No. 4 is in the first place in each case (except with 75 mg. per kilo) when Nos. 7 and 3 are shown to cause convulsions before No. 4. In such cases as this, it is due we think to slight differences in the rate of absorption.

RÉSUMÉ AND CONCLUSIONS

The results of the various methods are in general agreement that the anesthetic power of an homologous series increases with the molecular weight. The results recorded in this investigation do not always agree with this statement, but there is little reason to doubt the validity of it. The variations are to be explained mainly on variations in the rate of absorption. In such cases if a sufficiently large number of experiments were carried out the average would follow the general rule. Where the conditions can be more rigidly controlled as in the conjunctival sac, and in the determination of the phenolic coefficient, increasing action with increasing molecular weight is obvious. The toxicity as determined by injecting a solution of the drug into the peritoneal cavity is less than cocaine and somewhat greater than novocaine. The increase in toxicity, however, is less than the anesthetic action. As a result of this study Tables IV and V, the following numbers would seem worthy of clinical investigation.

A or ethyl series—III, IV, V, and VII.

B or propyl series—II, IV, and V.

PHENOLIC COEFFICIENT*

BY M. B. BURNS, B.S., AND N. BULLOCK, B.S., CHICAGO

THE phenolic coefficient was obtained by putting typhoid bacilli in a strength of the solution, and determining how soon death took place in this solution.

TABLE I

PHENOLIC COEFFICIENT (ARRANGED IN ORDER OF EFFICIENCY)

DRUG	PERCENTAGE OF SOLUTION NECESSARY TO KILL	TIME NECESSARY TO KILL TYPHOID
A 2	1	9 hr.
A 3	1	5 "
A 5	1	4½ "
B 2	1	3 "
B 1	1	2½ "
B 3	1	1½ "
A 7	1	45 min.
Phenol	1	5 "
A 4	1	2½ "
B 5	0.5	5 "
B 4	0.33	5 "
B 6	0.20	5 "
A 6	0.15	5 "

The order of toxicity of the ethyl and propyl series is as follows:

ETHYL SERIES	PROPYL SERIES
2	2
3	1
5	3
7	5
4	4
6	6

*Received for publication, March 28, 1925.

A great number of determinations were made, and Table I gives the final results. The determinations were made as follows:

Five c.c. of the anesthetic solution were inoculated with one loopful of a twenty-four-hour growth of typhoid, and this was repeated with various dilutions until the minimal strength which would kill typhoid growth was determined. When no growth on broth was obtained by reinoculation from this solution, it was considered that the limit had been reached. A large series was determined, and in Table I the minimal lethal concentration with the time necessary for the germs to sojourn in this solution was recorded. The aim was to determine the time of death in 1 per cent strength, but some of the solutions were so active that dilutions weaker than this had to be made in order to get the relative toxicity. Table I is self-explanatory and gives the order of strength of the toxicity of these drugs.

A FILTERING CYLINDER AND CULTURE TUBE FOR BACTERIOLOGIC WORK*

By Eleeza Ablahadian, B.S., Glendale, Cal.

THE customary method of *cotton plugging* flasks, test tubes, burettes, etc., is by wrapping the cotton in a piece of gauze and plugging the flask which is to be sterilized and saved for later use. When flasks containing solutions are subsequently opened, some cotton fibers often float around and fall into the solution. My first thought was to turn a beaker upside down over the mouth of the flask, without cotton. This method is satisfactory if the beaker fits well, and the flask is not to be shaken much. (Fig. 2.)

It is, of course, a development of the Petri dish idea; but, it is not always easy to find beakers that will fit the flasks.

I therefore moved the cotton from the mouth of the flask to the neck. (Fig. 3.) If the cotton is wrapped tightly around the neck of the flask, and if the beaker is clean and dry to begin with, during sterilization the cotton will stick to the flask, but not to the beaker, which is fitted over the cotton rather loosely. In opening the flask, remove the beaker carefully, holding it mouth down, pour the solution out, pass the beaker over a flame, flame the mouth of the flask without touching the cotton, and replace the beaker. There will be no scorched bits of cotton floating around, sticking to the mouth of the flask, or falling into the solution.

Using the Petri dish idea again, I devised a *culture test tube.* (Fig. 4.) This test tube has a groove about three-fourths of an inch from the mouth. Cotton is wrapped around the groove and a glass cap is inverted over the mouth of the test tube, covering the cotton. The cap has a flat knob, so that when necessary to open the test tube, it may be held between the left thumb and forefinger, while the test tube is held between the fore and middle fingers.

*From the Research Laboratory, Harrower Foundation Clinic, Glendale, Calif.
Received for publication, July 20, 1925.

The right hand manipulates the platinum rod. The cap may be sterilized before and after operation, and replaced. Cultures keep well in these tubes.

THE FILTERING CYLINDER

Those who do a great deal of vacuum filtering, using small quantities at a time, know what a troublesome process it is. Usually, a sterile test tube is placed inside the filtering flask to collect the filtrate. It is troublesome to

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 4+5

FIG. 5

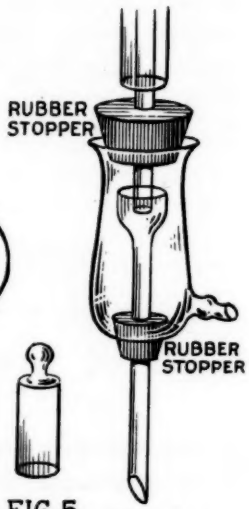

FIG. 6

stop the operation, take everything apart, remove the test tube when full, empty it, replace, and start filtering again. At the same time, there is the risk of contaminating the filtrate.

With a filtering cylinder, once the filter is placed and vacuum started, the process can go on without interruption. If a water pump is used, the cylinder takes care of the back-flow, and there is no danger of water getting into the filtrate. The receiving flask or bottle, placed at point "A," can be of any size, and may be removed without touching the rest of the apparatus.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Coca, A. F., and Milford, E. L.: The Preparation of Fluid Extracts and Solutions for Use in the Diagnosis and Treatment of Atopic Conditions. Jour. Immunol., March, 1925, x, ii, 555.

Coca and Milford report a continuation of their studies in this subject, this being the seventeenth paper of a series.

The paper, though brief, contains a wealth of information and should be in the files of every worker engaged or interested in this work.

They find that, except for pollen extracts, dialysis offers a ready means of purifying many of the extracts with resultant increase in activity and stability.

Dialysis is always carried out against the same solution as was used for extraction, using the largest caps supplied by the Capes-Viscose Inc. Co. (132 Madison Ave., New York). Perfect antisepsis can be secured by a little toluol inside the cap. The process is carried out at room temperature, the outside fluid being changed once or twice daily for, usually, six days.

For all materials except pollens the extracting fluid is prepared as follows:

Two stock solutions are made up:

I.	KH_2PO_4	3.63 grams
	Na_2HPO_4	14.31 grams
	NaCl	50 grams
	Distilled water to make 1000 c.c.	
II.	Carbolic acid 4 per cent.	

To equal parts of these fluids add four parts of distilled water. The resultant mixture is the buffered saline extracting fluid (B.S.), and has a PH of 7.0. It can be boiled or autoclaved without appreciable change in reaction.

The phosphate salts can be obtained from the J. T. Baker Co. and it is important to specify that they are to be used in the preparation of buffered solutions.

For pollen extractions Coca's original fluid is still used.

The formula for this (Jour. Immunol., March, 1922, vii, No. 2, p. 163) is:

Sodium chloride	0.5 per cent.
Sodium bicarbonate in such concentration that 10 c.c. of the fluid equaled about	3 c.c. of n/10 alkali.
Carbolic acid in final concentration of	0.4 per cent.

Solution should be made without the use of heat or excessive shaking.

For the cleansing of Berkefeld filters so that a neutral reaction results the following procedure was found satisfactory:

Mechanical cleansing with a "not too stiff bristled handbrush" is followed by immediate boiling for fifteen minutes in 2 per cent washing soda. Rinse in tap water and again boil. Ten to twenty filters are then boiled in 2 liters of distilled water to which has been added 50 c.c. of a 1:100 dilution of concentrated HCl. After two further boilings in plain distilled water, the filters are set up as filtering flasks and 300 to 400 c.c. of distilled water drawn through each. They are then wrapped in muslin and autoclaved.

Detailed and minute directions are given for many preparations. They do not lend themselves well to condensation or abstraction and the original paper should be consulted for the method of treatment of pollens, horse dander, feathers, rabbit, guinea pig, goat, cat, and dog hair, cow dander, nuts, seeds, and spices.

A new observation is recorded concerning milk as a result of which the authors no longer separate the proteins as test materials. It was found that raw, fresh milk from which the cream had been thoroughly separated by a Sharpless centrifuge could be filtered through a Berkefeld (N) filter. Such filtered milk remains stable for two to three months and is used for testing in 1:10 to 1:100 dilutions.

The methods described in the paper have given extracts remaining stable for at least a year in the majority of instances.

Following the observation that a quantity of suspended material, assumed to be largely desquamated epithelial cells, was present in the washings of feathers, and the substantiation of the assumption that the active substances were contained in these scales, the authors now routinely use an extract of this material in testing feather sensitive individuals.

In the preparation of hair extracts only the suspended particles in the ether washings are used, the hair being discarded.

The paper should be consulted in its original form for a full appreciation of the data contained.

Seyderhelm, R.: The Testing of the Vitality of Isolated Cells by Means of Colloidal Dyes. Deutsch. med. Wchnschr., Jan. 30, 1925, li, 180.

Cellular death is marked by the nuclear absorption of a colloidal dye; injured cells permit the slow passage of the dye through their injured membrane.

A mixture of congo red and trypan blue (strength not stated) has been applied by the author to the study of urinary sediments.

After centrifugation a drop of the sediment and a drop of the mixed dye solutions are examined under a cover-glass.

Leucocytes from inflammatory foci remain unstained; pus cells (dead leucocytes) are stained red or with a tinge of violet. Vaginal and bladder epithelium are usually mottled. Casts show marked variations, sometimes dependent upon the vitality of adherent cells.

Waxy casts are deep blue; hyalin, variable. Mucus is red.

It is suggested that this "staining of degeneration" could be extended to other materials as exudates, cerebrospinal fluid, etc.

Cornwall, L. H., and Peck, S. M.: Three Cases of Granuloma Inguinale with an Investigation Concerning the Etiology. Proc. New York Path. Soc., xxxv, 1-5, p. 43.

The authors report that the organism demonstrated in their cases presents a very characteristic appearance on Sabauraud's medium—4 per cent maltose peptone agar. The size is variable—1 to 6 micra long by 0.5 micron wide, one end rounded and the other tapering to a point. With the Gram stain there is a definite cell membrane and some pleochromism. The cytoplasm contains from 1 to 5 purplish granular bodies. Occasional bipolar bodies or beaded forms are seen and oval, circular, or polyhedral forms are encountered. These characteristics are lost after 180 hours on this medium and are restored with great difficulty, if at all, by subcultures which revert to the diplobacilli, diplococci, bacillary, and coccoid forms usually seen on plain agar.

The authors consider the organism entirely distinct from B. mucosus capsulatus.

Morris, W. H.: The Value of Erythrocyte Sedimentation Determinations in Pulmonary Tuberculosis. Am. Rev. Tuber., December, 1924, x, 431.

The technic adopted by the author is as follows:

Blood is collected in a syringe containing sufficient 3.8 per cent sodium citrate to give a 1:4 dilution of the blood. The blood is placed in small Wassermann tubes.

Calibrated 1 c.c. pipettes are filled to the 1 c.c. mark, stoppered with a bored-out rubber cork, and placed in racks at room temperature. Readings are made at one, two, and twenty-four hours, representing percentages of the total volume of the blood.

Normal findings in the male were 1 to 5 per cent; in the female, 5 to 10 per cent.

Studies were made on 150 patients in varying stages of tuberculosis. The conclusions were:

1. Sedimentation velocity is increased in active pulmonary tuberculosis.
2. There is no constant relation between the extent of the lesion and the velocity of sedimentation.
3. The test offers a valuable estimate of the degree of activity.
4. The test has little value in diagnosis.

Zecker, T. T. and Goodell, H.: The Sedimentation Rate of Erythrocytes. Am. Jour. Med. Sc., February, 1925, clix, 209.

Two centimeters of 3 per cent sodium citrate are placed in a 15 c.c. graduated centrifuge tube and blood added to the 10 c.c. mark.

The tube is inverted and allowed to stand and the height of the red cells read at one hour. If the plasma is slightly higher or lower than the mark, the volume of red cells is corrected accordingly.

Studies were made of 125 cases. Readings were as follows:

Tuberculosis and acute inflammatory conditions, 3 to 6 c.c.; malignant tumors, 2 to 7 c.c.; pregnancy, 3 to 8 c.c.; all other conditions, 4.5 to 9.8 c.c.

Average normal readings were 7.1 c.c.

The authors recommend serial determinations and emphasize the necessity for correlation of the test with the other clinical and laboratory findings.

Julianelle, L. A., and Pons, C. A.: A Study of the Serologic Reactions Associated with Experimental Plethora and Plethoric Anemia. Jour. Clin. Investig., August, 1925, i, vi, 519.

Experimental studies have shown that following the artificial production of plethora an anemia occurs the mechanism of which has not been explained.

Explanations considered probable embrace: (a) the development of isohemolysins, and (b) destruction of transfused blood by increased activity of hemophages or through an increase in the number of such cells.

The authors report studies devoted to the demonstration of these possibilities.

Artificial plethora was induced in rabbits by the daily injection of rabbit blood (mostly citrated).

Incompatibility was never encountered. Plethora occurred, as a rule, in four to seven days and varied from 30 to 100 per cent increase in cells and from 20 to 50 per cent increase in hemoglobin. A gradual fall then occurred in spite of repeated transfusions, the fall stopping in some cases when reduction reached the normal level, in others progressing to a true anemia.

The red cells dropped before the hemoglobin, the graph being generally a straight line. The hemoglobin graph was step-like.

The reticulocytes varied inversely as the red cell count. The leucocytes fluctuated irregularly. Plethora was associated with lymphocytosis; anemia with a decrease in lymphocytes in favor of the neutrophiles. Rarer forms of white cells were infrequent.

The changes were the same regardless of whether or not the blood was citrated.

Marked congestion of the spleen and liver; intra- and extracellular hemosiderin deposits in large amount; endothelial hyperplasia; absence of changes in the lymph follicles; congestion and casts in the kidneys; and engorgement of capillaries with cellular debris and hemosiderin were the salient autopsy and microscopic findings.

Isohemolysins were not demonstrated either in vitro or in vivo.

Isohemagglutinins were found in half the animals but bore no relation to the degree of blood destruction.

Evidence of phagocytic or hemophagic activity was not demonstrated in fixed tissues. There was no evidence of any potential grouping in the rabbit blood.

The explanation of postplethoric anemia awaits further investigations.

Pilot, I., and Meyer, K. A.: Fusiform Bacilli and Spirochetes XII: Occurrence in Gangrenous Lesions of the Finger. Arch. Dermat. and Syph., December, 1925, vi, 837.

The previously reported studies of Pilot and Pilot and Davis have demonstrated the organisms first associated with Vincent's angina as very widespread invaders responsible for a variety of pathologic conditions.

In the present report Pilot and Meyer report their occurrence as causative agents in gangrene of the finger in a patient addicted to nail-biting, the organisms undoubtedly having been transferred by this habit.

The condition was treated successfully by neoarsphenamine.

The article is illustrated.

Schobl, O., and Ramirez, J.: The Fallacy of the Test for Lactose Fermenters as an Indicator of Fecal Pollution of Waters. Philippine Jour. Med. Sc., July, 1925, xxvii, 317.

The authors endeavored to determine, in view of the importance attached to lactose fermenters as an indication of fecal pollution in water, whether or not these forms are indisputably indicative of fecal contamination and, also, whether the representatives of the B. coli group are limited to human and animal excreta predominantly or if B. coli of human origin can be differentiated from B. coli of animal origin.

Studies were made upon well waters in Manila in the course of which it was found that parts of the pump which were constructed of organic material, such as hemp and leather washers, harbored large numbers of lactose fermenters. The same findings were noted in the bacteriologic examination of fresh, unused parts.

Other examinations disclosed lactose fermenters as nearly ubiquitous, being present in dust, grass, leaves, and shrubs.

Their presence in water, therefore, cannot be taken as final evidence of fecal pollution and should not serve as the sole basis for the condemnation of a water supply.

Specimens of feces were examined from various sources: fish, frogs, shrimp, water insects, horse, goat, carabao, rabbit, guinea pig, white mice and rats, rooster, owl, and man—in the endeavor, which was unsuccessful, to find some points serving to differentiate colon bacilli of animal and human origin.

The conclusions formed from the work follow:

1. Lactose fermenters as a group cannot be considered as an indication of fecal pollution.

2. True B. coli can be differentiated by the type of colonies on eosin-methylene-blue-lactose-agar plates.

3. There are no criteria for the differentiation of B. coli of human origin from those of animal origin.

4. The pollution of water by B. coli from the feces of fish or water-living animals is unlikely under ordinary circumstances.

Fischel, K.: The Suspension Stability Rate of Erythrocytes in Pulmonary Tuberculosis and Its Significance in Artificial Pneumothorax. Am. Rev. Tuber., January, 1925, x, 606.

Commenting upon the variations in technic associated with this procedure, the author proposes a method of calculating the reading which eliminates the necessity for specially calibrated tubes, etc.

It is based upon the principle of constant height rather than constant quantity of blood as suggested by Westergren.

Tubes of $\frac{2}{8}$ to $\frac{3}{16}$ inch diameter are used and the results expressed by calculating the height of the clear column in percentage of the whole suspension column.

A 2 c.c. syringe containing 0.4 c.c. of 3.8 per cent sodium citrate is filled with blood to the 2 c.c. mark. The syringe is inverted and the blood placed in the tube, which must be clean and dry. The tube is placed in a rack at room temperature and readings are made at one, two, six, and twenty-four hours.

The height of the whole column in millimeters is divided into the height of the plasma column, the quotient giving the percentage reading.

The paper contains numerous tables and graphs and, on the whole, confirms the investigations of previous workers in similar conditions.

Fraser, J. F.: Mycosis Fungoides, Its Relation to Leukemia and Lymphosarcoma. Arch. Dermat. and Syph., December, 1925, xii, No. 6, 814.

Fraser adds three cases to the growing number of instances in which mycosis fungoides appears to have been associated with neoplastic changes in the tissues.

In one case there was the development of a leucemic blood picture, in another the morphologic picture of lymphosarcoma. The third case was in a South American squirrel in which the lesions were identical with Sternberg's leucosarcoma.

The name reticulum cell sarcoma is suggested to replace lymphosarcoma in designating the lesions of mycosis fungoides on the assumption that the lesion has its origin in the reticulum cells of the papillary layer (reticulo-endothelial septum of the skin).

Roman, B., and Lapp, C. M.: Lesions of the Central Nervous System in Canine Distemper. Buffalo Gen. Hosp. Bull., September, 1925, iii, No. 2, p. 40.

The paper, which is excellently illustrated with numerous plates, reports the histologic examination of the brain and spinal cord in 29 cases of distemper.

The lesions, which were present in 19 animals, were in the main all similar and consisted of a definite inflammatory reaction of the pia mater in the region of the pons, medulla, and also in the cerebellum.

The infiltrating cells were mainly lymphocytic. Marked degenerative changes were found in the nerve cells, especially in the cord.

Stains for bactria were uniformly negative.

In brief, there was a disseminated polio- and leucomyelitis and areas of encephalitis associated with a patchy leptomenigitis with focal necrosis hemorrhages, and extensive degeneration of the nerve cells.

The authors believe that the work concerning the etiologic rôle of B. bronchisepticus requires revision.

Osgood, H.: Hemagglutinative Properties of Diphtheria Antitoxin. Buffalo Gen. Hosp. Bull., September, 1925, iii, No. 2, p. 37.

This report comprises the results of the examination of 26 specimens of stock antitoxin in which tests were made against the erythrocytes of a single individual for the presence of hemagglutinins in the antitoxin sera.

Agglutinins against human erythrocytes were found in all samples but this property, though relatively strong in fresh, unaged samples, did not appear to be strong enough in lots aged and issued for therapeutic use to cause trouble when intravenously injected.

Myers, C. N., and Cornwall, L. H.: Normal Arsenic and Its Significance from the Point of View of Legal Medicine. Am. Jour. Syph., October, 1925, xi, No. 3, p. 647.

As recalled by the authors, in an interesting introductory review, death by poisoning, either suicide or murder, and the use of arsenic as a lethal agent, are referred to in the earliest known medical literature.

Modern investigations have also led to the increasing use of this drug and its derivatives in the treatment of a variety of diseases. In as much as the chemical detection of arsenic in the body tissues is often of great forensic importance, it is essential to determine if its appearance is normal or entirely adventitious.

Prior to the work of Gautier in 1889 arsenic was regarded as an entirely adventitious substance in human tissues and so, upon occasion, of marked medicolegal significance.

Gautier reported its presence as a normal constituent of human tissues but these findings were disputed and disclaimed and have not been given much importance from a forensic

point of view and, in order to confute the criticisms and objections which have been made of various reported investigations on this subject, Myers and Cornwall report further investigations the results of which are indicated below.

Arsenic is present in a large variety of foods, in candy, and may also be taken into the body by external or internal medication.

In small amounts—not to exceed 0.3 mg. per 100 c.c.—arsenic is a normal constituent of human blood as determined by the examination of 200 individuals.

The presence of 0.4 mg. per 100 c.c. of dried specimen may be taken as a normal value for human urine.

Arsenic may be regarded as a normal constituent of human hair, but is not found in human milk unless the patient is under medication, when appreciable amounts of arsenic may pass over into the body of the nursing infant.

A large number of infants examined showed the presence of arsenic in all important viscera, the amounts in the brain and cord being occasionally high. As the age of the child increased, the amount of arsenic decreased.

Cases of pneumonia and cardiac disease in adults showed arsenic in large amounts in the lungs and heart.

It is obvious from these findings that the Marsh mirror test for the presence of arsenic in human tissues must be conservatively interpreted in medicolegal examinations.

The article contains numerous detailed tables.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Practical Bacteriology**

"AN Introduction to Practical Bacteriology as Applied to Medicine and Public Health" is presented as a guide to bacteriologic laboratory work for students and practitioners of medicine. This book is devoted primarily to discussions of those pathogenic bacteria which play a part in the causation of human disease. The biologic classification does not conform entirely to that adopted by the Society of American Bacteriologists, but this in no way obscures the presentation. The book follows the usual textbook program including the general biology of microorganisms, their relationship to immunity, the use of the microscope and preparation of culture media, staining methods, and animal inoculation. There follows a section on special bacteriologic methods, such as blood cultures, Widal reaction, agglutination, vaccine preparation, examination of milk and water, and of various germicides. The second section deals with bacteriologic diagnosis and is particularly to be recommended as an instrument for reference in which most of the desired information may be found boiled down into a systematic presentation.

Essentials of Immunology†

WRITTEN primarily as a manual for medical students, this work should be of value to all laboratory workers dealing with immunologic methods. The author gives a relatively brief didactic recapitulation of the general subject of immunity, dealing particularly with its practical aspects. It is a pleasure to see, at last, one book which assigns to a less important place some of the older concepts which have been supplanted but still dominate medical literature. This applies for example, to the Ehrlich side-chain theory of immunity, which, while having been of inestimable value in directing research, has at the same time been accepted too literally because of the simplicity of its pictorial presentation. The author has introduced some new nomenclature.

The second portion contains a series of exercises for students, describing in detail the application of the Schick test, the Dick test, diphtheria toxin and antitoxin standardization, absorption, titration and agglutination experiments, identification of human blood, desensitization, complement fixation, and vaccination.

*Practical Bacteriology. By Drs. T. J. Mackie, D.P.H. (Oxford), and J. E. McCartney, M.D., D.Sc. (Edin.). Cloth. Pp. 297. Price $3.50. William Wood & Co., N. Y., 1925.

†Essentials of Immunology for Medical Students. By Arthur F. Coca, M.D., Professor of Immunology in Cornell University Medical College. Pp. 194. Cloth. Price $3.50. Williams & Wilkins Co., Baltimore, 1925.

*Clinical Laboratory Medicine**

THE purpose of this book, as stated in the foreword, is "to present laboratory medicine through its clinical interpretation."

It is admittedly not intended for the research or advanced laboratory worker but for the student and practitioner.

The task which these authors have set themselves is one of some difficulty. So manifold are the resources of clinical pathology, and so hastily must they be reviewed in the crowded medical curriculum of the day that, it must be confessed, many students carry away the impression that it is the *test* which is of predominant importance rather than its *interpretation* and application to the patient.

Many overlook the fact that the division between clinical and laboratory medicine, so-called, is artificial and arbitrary; that the terms clinician and clinical pathologist should indicate, not two diverse and unrelated varieties of practitioner, but merely the particular phases of the medical art in which each has a compelling interest.

The first step in the management of disease is its recognition, its diagnosis, and to the formation of a diagnosis, the resources of the clinical laboratory may be so utilized as to bring much information that is of value and, sometimes, of essential importance. To apply this information and to utilize it to its full worth, the student and practitioner must realize that it constitutes only *a part* of the examination of the patient.

The manifestations of disease, in essence, are the manifestations of disturbances of function, the character and degree of which are many times best determined in the clinical laboratory. The ultimate *significance* of the abnormalities thus discovered depends upon the correlation of the findings thus obtained with all the other available data.

To present this subject in this way is a matter of some difficulty. If the treatise is comprehensive, it is apt to confuse the practitioner by the variety of methods and data presented; if too condensed, it fails, perhaps, to enlighten.

The authors have chosen a middle course.

The book appears to be essentially a manual of the methods taught to the students of the Long Island Medical College. In the main, the methods are well chosen and clearly described in detail and each is followed by some discussion of its clinical interpretation and application. At times this could be expanded with benefit.

There is an extensive section on blood chemistry embodying the technic of Folin and Wu and a very clear discussion of hydrogen ions and their determination. The laboratory criteria of acidosis are well presented. Clinical methods for blood transfusion, the therapeutic uses of convalescent serum in certain diseases, and the application of the Schick and Dick tests are discussed. The section on blood sugar does not note the practical papers of John on its clinical interpretation.

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*Clinical Laboratory Medicine, H. M. Feinblatt, M.D., and A. E. Eggerth, A.B., A.M. Pp. 411. Wm. Wood and Co., New York, $5.00.

The methods in general are modern. In the determination of red cell fragility, however, the relatively cumbersome technic of La Roche is described without mention of the rapid and simple method devised by Sanford and Giffin. The Wassermann technic described is that of Walker and Swift with a 0.4 per cent cholesterinized antigen and a primary incubation of one hour at 38° C. In view of the very extensive studies which have been conducted on this subject, no mention of which is made, this cannot be said to represent the latest consensus of serologists.

Under diacetic acid the Gerhard test is given without mention of the false reactions encountered when sodium bicarbonate is administered.

The proof reading and general preparation of the book are good and the illustrations apt.

References are placed directly in the body of the text "to facilitate rapid access." This innovation seems of dubious value. To the reviewer it seems to furnish an annoying break in the continuity of the text and an interference to smooth reading. It is also noted that the majority of the references cited seem to cover the older literature, few being later than 1919.

The book will serve a useful purpose for those to whom it is addressed.

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EDITORIALS

Clinical Electrocardiography and Recent Advances in Cardiac Physiology

CLINICAL electrocardiography, the laboratory method of precision par excellence, has come into its own well-merited and well-founded eminence through the painstaking studies of a long line of cardiac physiologists. Einthoven and Lewis have established firmly the foundation of modern cardiology. The clear conception of the mechanism of the heart beat in health and in disease has been made possible by the vigorous application of the method of the string galvanometer to the study of clinical cases of heart disease. Proficiency in bedside diagnosis and treatment of cardiac conditions is dependent upon experience that has been and continues to be frequently confirmed by electrocardiographic studies. The field of usefulness of the method is more and more generally appreciated. There are but few internists who do not realize the value of the method, in the detection and the determination of the exact type of conduction disturbances, such as a prolonged a-v. interval or a higher degree of block in the His bundle or of block in a branch of the bundle; in the recognition of auricular flutter and at times even of auricular fibrillation

with certainty; in the identification of the point of origin of a paroxysmal tachycardia; in the interpretation of murmurs and in changes or in a fixation of the electrical axis as evidences of asymmetrical hypertrophy or adhesive mediastinopericarditis; and, finally, in the gauging of the effects of therapeutic agents, especially drugs on the mechanism of the heart beat.

However, we have not arrived at this advanced stage of proficiency of the method at one jump. The rise has been by no means meteoric, and the progress, or advance henceforth will most probably be slow.

Our old theory of the development and propagation of the excitatory difference of potential and the resulting mechanical response by a rhythmic anabolic chemicophysical process in the specialized and ordinary cardiac tissues has been amplified and extended by the recent work of Andrus and Carter. These investigators have perfused the hearts of terrapins and dogs with isotonic solutions of known P_H value and have been able to "speed up" and to slow down, to stop and to start again the development of the excitatory process and its propagation by changing the P_H of the perfusion fluid. They conclude that the rhythm and rate of conduction in cardiac tissue depends to a great extent if not entirely upon the hydrogen-ion concentration of the blood and lymph. They consider the generation of the difference of potential (the negativity) to be due to a disturbance of ionic equilibrium between the cell contents, colloids and salts, sodium, calcium and potassium, on the inside of the semipermeable cell membrane and the surrounding tissue fluid of similar composition but of different concentration on the outside. Lillie's conception of the process of conduction depending upon the excitation of the adjacent area by the action current developed at the point of primary excitation is accepted to explain the propagation of the excitatory process in the heart, this in turn being determined by the difference of potential existing at the point of stimulation and by the permeability of the cell membrane, both of which circumstances depend upon the relative ionic concentration within and without the cell, which is finally in the last analysis also controlled by the hydrogen-ion concentrations. Disturbances in rhythm are thus the result of changes in P_H concentration not only in fluid, as Andrus and Carter point out, but in the tissues, the specialized and the ordinary heart muscle cells also.

Ashman in a recent important fundamental study compressed the auricular muscle and junctional tissues of the turtle heart, thus changing the P_H toward acid values in these tissues, and studied graphically the conductivity. He found (1) that following the transmission of an impulse through the compressed muscle there was at first an interval during which a second impulse if discharged from the auricle would fail to reach the ventricle; this was followed by a period during which the impulse was transmitted slowly, then more rapidly until finally the resting conductivity was regained. The course of the recovery was along a smooth curve. (2) Blocked impulses were found to influence the rapidity of conduction of subsequent impulses. The later the blocked impulse falls in the cycle, the more it prolongs the conduction time of the subsequent transmitted impulse. (3) Evidence suggesting slower recovery following blocked impulse was also given. (4) The interval of

the blocked impulse was considered by Ashman to include the earliest part of the relative refractory period.

Ashman in his second paper, also dealing with recovery of conductivity in compressed cardiac muscle, demonstrated a *supernormal* phase in conductivity. This phenomenon appeared under conditions which contributed to fatigue of the muscle, but fatigue effects were obtained without evidence of a supernormal, but never supernormal without fatigue effects. The recovery curves returning rapidly to a normal or resting conductivity passed beyond the base line for a greater or lesser distance and then gradually returned to the line representing the resting conductivity. Thus an impulse arriving at the compressed region four or five seconds after transmission of a previous impulse was actually transmitted more rapidly and in many cases much more rapidly than one delivered after ten, twenty or thirty seconds. With an increase in the degree of compression a stage might have been reached in which all impulses would be blocked excepting those falling on or near the crest of the supernormal phase. Lewis, and Wolferth have each reported a clinical case illustrating this phenomenon.

There was considerable difficulty, under the conditions of these experiments, in determining the exact form of the supernormal recovery curve. This was due to two phenomena. The first was the appearance, after rest, of a treppe or staircase in conductivity which caused the conduction times for a short series of impulses, each delivered during the supernormal phase left by its predecessor, to show a progressive decrease in length. The second was the appearance during continuous activity of cumulative fatigue, and the recovery from this fatigue which occurred after a minute or two. Ashman therefore obtained curves which showed, following the post-supernormal depression of conductivity, a gradual return of conductivity with prolonged rest. As a consequence in a typical case a degree of block might often be produced which would cause impulses arriving within 2.5 seconds following a previous impulse to be blocked; from 2.5 seconds to about 7 or 8 seconds, to be transmitted; from 8 to about 25 seconds, to be blocked; and after 25 seconds, to be transmitted. The success or failure of each individual impulse was also conditioned by the degree of cumulative fatigue or the antecedent appearance of a treppe in conductivity.

The treppe was best seen after an intermediate rest period, that is, before much recovery from fatigue had occurred. As a consequence of the treppe phenomenon when the compression was right and when a series of impulses at four second intervals arrived after a 20 second rest, the first impulse to be transmitted to the ventricle might be the third, fourth or even the fifth of the series. If the impulses continued to arrive at this rate several more may be transmitted in succession until a partial block developed as a result of fatigue. The block was ushered in by a prolongation of the conduction time. If, under these same conditions, a much longer rest was allowed before the series of impulses at four-second intervals was delivered at the compressed region, the first impulse might be transmitted most rapidly, the second almost as rapidly, the third somewhat more slowly, the fourth or fifth much more slowly until a partial block developed. Here the treppe was represented, not by a decrease

in the a-v. interval, but by the failure of the fatigue effects to appear as quickly as they otherwise would.

This relation is perhaps of importance for its bearing upon Iliescu and Sebastiani's criticism of the Kaufmann and Rothberger theory of parasystole (*Heart*, 1923, x, 101). Iliescu and Sebastiani state that the time relations of the transmission intervals postulated by Kaufmann and Rothberger are not those actually seen in partial block. If a supernormal phase were present, the time relations in question might have been possible.

It is fundamental work of this type that extends the bounds of our experience and blazes the way for further experimental and clinical investigation, that will add to the sum total of our knowledge.

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—*G. R. H.*

The Cleveland Meeting of the Federation of American Societies for Experimental Biology

THE annual meeting of the Federation of American Societies for Experimental Biology was held at Cleveland on December 28, 29 and 30, 1925. The yearly meetings of this organization may well be considered as the marking of milestones in the progress of American medical science. For, as a rule, these meetings really represent a summing up of practically all of the fundamental and basic discoveries made in biologic fields in this country during the preceding twelve months.

The federation is composed of four essentially independent societies, the Physiological Society, the Society of Biological Chemists, Inc., the Society for Pharmacology and Experimental Therapeutics and the Society for Experimental Pathology. In 1913 these independent societies formed the federation and they have met together each year since that date. It would perhaps be to the advantage of the federation if other scientific organizations such as the anatomists, the zoologists or the anesthetists might meet each year with the federation. This would undoubtedly result in a considerable financial gain to the attending members of the federation in those instances in which less than two hundred and fifty railroad tickets are sold to members visiting the meetings. It happened, however, this year for the first time, that the federation, meeting alone (*and in the west*), had in attendance a sufficient number of visiting members to require something more than three hundred railroad tickets and therefore the usual reduction in rates to one and a half fares for the round trip was allowed by the railroads. This small financial saving was undoubtedly of considerable importance to the very group of young men whom science most needs in this country at the present time. For it requires only a glance at the published programs of the federation to see that many of the new things presented, and, indeed, often discoveries of great and fundamental importance are brought forward by men whose names are new in the federa-

tion—men indeed whom only a year or so ago the members of the admissions committees were often weighing with judicial seriousness in the infallible scales of the past, the now and the hereafter to determine whether or not, if these men were admitted to the societies, they might lower the dignity of the organization. And, being human, it is probable that these committees have occasionally failed to read the scales correctly.

To the officers and faculty, and especially to the local committee of the medical school of the Western Reserve University are due the sincere thanks and the hearty praise of the whole federation for the splendid success of the entire series of meetings. The magnificent buildings and the splendid equipment of the new medical school undoubtedly served as a special attraction in inducing many members to attend the Cleveland meetings. A brief inspection of this splendid new group of buildings—a typical, modern, American medical plant—is sufficient to remind one again that all of the first-class medical schools in this country are rapidly approaching a fairly common standard so far as buildings, equipment, clinical material and, in the main, financial backing, are concerned. Wherefore we shall again revert to our former test for the standing and prominence of any given medical school. And the best school will again become the one that has the greatest number and the highest type of men on its faculty.

Ohio again touched a spot near to the hearts of the federation members, when, on Monday evening, December 28, the Western Reserve University tendered a splendid complimentary dinner to the members of the federation *and their wives* at the Mid-day Club. This is almost the first time that any group or organization in entertaining the entire federation has extended a special invitation to the wives of the members to attend any phase of the annual meetings, and the incident speaks most highly for the delightfully sympathetic and hospitable manner in which the university and the medical faculty put forth every effort to make the occasion a most enjoyable one for the whole federation. This will perhaps remind some of the older members of former times when the highest business ideal of American universities was to pay each man on the faculty a salary which was barely sufficient to support him (so long as he did not need the services of a doctor), and it was tacitly assumed among members of the societies that, as a matter of courtesy, no reference should be made to the possibility that some of the braver and more heroic members might be attempting to support wives in addition to themselves. It is a harbinger of the times that, in striking contrast to the old days, a goodly number of ladies, perhaps more than forty, attended the Cleveland meeting, and their names appeared on the programs either as sole authors, or as co-authors, of some thirty-six papers and demonstrations.

As mentioned above the federation is composed of four constituent societies. It is the custom that, in rotation through four yearly periods, the president and the secretary of each constituent society shall act as chairman and secretary respectively of the entire federation. In accordance with this rule Prof. A. J. Carlson of the University of Chicago and Prof. W. J. Meek of the University of Wisconsin served this year as general chairman and general secretary, respectively.

The first general session was held at the Hotel Statler on Monday morning, December 28. At this session the announcement of the deaths during the preceding year of Prof. A. W. Hewlett, of San Francisco, Prof. J. N. Langley, of Cambridge, England, Prof. Reynold A. Spaeth, of Baltimore, and Prof. Emeritus John Marshall, of Philadelphia, was made. As a mark of respect to the memory of these departed colleagues the members of the whole federation arose and stood with bowed heads for one minute.

This first session and also the final session on Wednesday afternoon were held at the Hotel Statler, an arrangement which proved exceedingly convenient from the standpoint of arrival and departure for the visiting members. All other sessions were held in the Western Reserve University Medical School buildings at 2109 Adelbert Road. A total of 209 papers were listed on the program to be read before the various societies. In addition to these 74 more were listed to be read by title. Most of Tuesday afternoon was given over to the presentation of demonstrations of which 30 were shown. The demonstrations constituted one of the most interesting and instructive features of the whole meeting, and each year more and more emphasis is placed on their development.

Dean Hamann of the Western Reserve University Medical School acted as toastmaster at the complimentary dinner given by the university on Monday evening. In a most deft and skilful manner (the dean is one of Cleveland's best known surgeons) the toastmaster opened the heart of the university in a cordial welcome to the federation. He introduced President Vinson who emphasized this welcome by stating that on a former occasion he had been placed as seventh man on a list of speakers all of whom had for their duty the welcoming of an organization which was then visiting Cleveland. The mere fact that President Vinson survived that experience is sufficient evidence to indicate that as second man only in welcoming the federation he did justice not only to himself and the federation but also added honor to his university. He further expressed the wish that scientific men might make themselves more aggressive and effective in combating the ignorance and the superstition which oppose modern scientific progress in general and the spirit of evolution in particular.

The next speaker presented by the toastmaster was Prof. Carlson who stated that it was only at the repeated, urgent request of the Cleveland local committee that he had at last agreed to prepare a speech for the Cleveland meeting. And when he began this task it appeared that he cast about for some desirable and inspiring subject, but the only really adequate and suitable material which he could find *in Chicago* was a series of jokes by Prof. A. S. Loevenhart of the Department of Pharmacology of the University of Wisconsin. These jokes, Prof. Carlson stated, he gathered up and "fumigated" (this may be the Chicago expression for *redistilled*) and finally condensed into an admirable speech. This incident indicates in a striking manner the very great dependence of present-day physiology upon pharmacology. After its completion the speech was mailed to the Cleveland committee for "expurgation," as Prof. Carlson had been warned that "Mayors, deans, university presidents, city managers, etc., all of whom could *talk*—whether they had anything to say or not"

—were expected to be present at the complimentary dinner. Wherefore it became the general chairman's duty to present the federation to these distinguished gentlemen in the very latest and most approved style of oratory. Unfortunately, however, according to reports, this speech was lost in the mails somewhere between Chicago and Cleveland. In the absence of this set speech Prof. Carlson was led to refer briefly to a few incidents of a dream which he had had the preceding night. In this dream it appeared that Prof. Carlson was approaching heaven on a pair of high stilts. From this exalted station he could observe not only the pathway leading up to the main entrance, but he could also look through a knothole in the high board fence and observe part of the great show going on within. While thus terrestially supported in celestial surroundings he was much pleased to observe Prof. A. P. Mathews and Prof. A. B. Macallum coming up the main pathway, arm in arm, and discussing Presbyterianism, predestination and electronic psychism of the soul, all in respect to precedence in the matter of entrance into heaven. And on looking through the knothole Prof. Carlson was delighted to see Dr. Cannon, Dr. Stewart, Dr. Rogoff and Dr. Crile all seated together at a lemonade stand according to the custom of their former existence. They were not happy, however, for Dr. Cannon was complaining that there were no emergencies in heaven, and hence no need for the adrenal glands or adrenaline.

The regular subscription dinner of the federation was held on Tuesday evening, December 29, at the Hotel Statler. There was a large attendance and addresses were made by Drs. Carlson, Auer, W. Brown, Meek and Sherman. Following this dinner the annual conference of biologic chemists was held at the Hotel Statler. The subjects discussed referred to the methods and arrangements which might be employed for the most successful teaching of biologic chemistry. This, Prof. Carlson stated, was the annual biochemic rodeo.

The weather was very severe in Cleveland during the greater part of the meetings. It is probable that this had a psychologic effect on the councils of the various societies, for at a general meeting of the federation council it was decided to hold the next annual meeting in April, 1927. This change to a warmer season has been discussed for many years past and the experiment will now be tried, at least for a single meeting. The place where the meeting will be held has not yet been decided.

At the elections officers for the ensuing year were selected as follows: for the Physiological Society, president, Joseph Erlanger, secretary, W. J. Meek; for the Biochemical Society, president, E. C. Kendall, secretary, F. C. Koch; for the Pharmacological Society, president, John Auer, secretary, E. D. Brown, and for the Pathological Society, president, Wade Brown, secretary, E. B. Krumbhaar.

The Cleveland daily papers discussed the work of the federation in a most admirable manner. Many important papers were discussed at length, and the value of scientific investigations and discoveries was well emphasized. At least one paper, the *Cleveland Plain Dealer,* in its issue of December 30, 1925, published splendid hand-drawn illustrations of the three handsomest members of the federation, Dr. Carlson of Chicago, Dr. Gesell of the University of Michigan, and Dr. Meek of the University of Wisconsin, all western men. The success

of the west in the beauty contest would appear to be another reason why a majority of the future meetings of the federation should be held in the west.

Poetry was quoted on the floor of the convention in only a single instance. This was by Prof. A. B. Macallum who recited two short but delightful little poems. The first was by O. W. Holmes, the second by one of our later poets. The latter poem, a rare, little, literary gem, emphasized over again the old advice that one should not attempt to beat a crook by playing the crook's own game.

Finally, as a matter of record, it may be noted in closing that the deliberations and activities of the Cleveland meeting included everything from Heaven to rodeos, from metaphysics to pathology, from banquets to ladies and from University presidents to skunks, which subjects may fairly well be considered as defining the limiting boundaries within which the intellectual activities of the federation were primarily confined during the year 1925.

—*D. E. J.*

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The Annual Meeting

The 1926 Convention of the American Society of Clinical Pathologists will be held in Dallas, Texas, April 15, 16, and 17, 1926.

Elaborate preparations are being made by our Texas members to give their colleagues a truly southern hospitable reception. Entertainment will be provided for the visitors and their wives. Drs. Moursund and Black have already called a conference of all the clinical pathologists of Texas at which plans were laid and arrangements perfected to make this meeting a memorable one in the annals of our organization.

Make Your Hotel Reservations Now!

In view of the great influx of physicians in Dallas for the American Medical Association meeting, hotel accommodations will necessarily be at a pre-

mium. Inasmuch as the A. S. C. P. meets during the week preceding the A. M. A. convention, there will be room for all our members during our own meeting, but unless reservations are made right now, the rooms cannot be held for those who desire to stay over the ensuing week.

The Baker Hotel has been reserved as headquarters for the A. S. C P. This hotel offers ideal meeting rooms for our purpose.

Scientific Papers

Judging from the titles and abstracts of the papers to be presented, the program will maintain the high level of excellence shown at our previous meetings. There is still an eleventh hour opportunity for our members who have been dilatory in submitting titles of their papers to send them to the secretary at once for inclusion in the program. The subject matter of the articles submitted promises to be fruitful of discussion and of interest and profit to the clinical pathologist.

All Clinical Pathologists Are Invited

It has become a tradition with the A. S. C. P. to invite all clinical pathologists to our annual conventions be they members or not. To this end about five hundred invitations have been sent out to nonmembers extending to them the facilities of the Society.

The Society maintains a Service Bureau for its members. Any clinical pathologist wishing to make a change should communicate with the Secretary.

Kindly report any change of address to the Secretary.